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High Initial Titres of Anti-Spike Antibodies following SARS-CoV-2 Infection is Associated with Faster Decay Rates at Four Months Follow-Up

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High Initial Titres of Anti-Spike Antibodies following SARS-CoV-2 Infection is Associated with Faster Decay Rates at Four Months Follow-Up

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Abstract

Objective

Dynamics of humoral immune responses to SARS-CoV-2 antigens following infection suggests an initial decay of antibody followed by subsequent stabilization. We aim to understand the longitudinal humoral responses to SARS-CoV-2 nucleocapsid (N) protein and spike (S) protein and to evaluate their correlation to clinical symptoms among healthcare workers (HCW).

Design

A prospective cross-sectional cohort study.

Setting

This study was conducted in New York City Public Hospital in the South Bronx, New York.

Participants

Healthcare Workers participated in Phase 1 (N=500) and Phase 2 (N=178) of our study and underwent both PCR and serology testing, in addition to online survey. Analysis was performed on the 178 participants that presented for both phases of the study.

Primary outcome measure

Data from both phases over four months was collected on HCW that underwent serial qualitative serology testing for anti-N antibody, quantitative MSH-ELISA to detect Receptor Binding Domain and full-length S reactive antibodies to measure the decay rate and stabilization of the titres for SARS-CoV-2 infection.

Results

Anti-N antibody positivity was 27% and anti-S positivity was 28% in Phase 1. In Phase 2 anti-S titres were higher in symptomatic than in asymptomatic positive subjects in Phase 1. Marginally higher titers were seen in asymptomatic compared to the symptomatic positive subgroup in Phase 2. A positive correlation was noted between age, number and duration of

53 symptoms, and Phase 1 anti-S antibody titre. A strong correlation was observed between
54 Phase 1 titers and decay of anti-S antibody titres between the two phases. Significant
55 correlation with rate of decay was also noted with fever, GI symptoms, and total number and
56 duration of COVID-19 symptoms.

57 Conclusions

58 Higher initial anti-S antibody titres were associated with larger number and longer duration
59 of symptoms as well as faster decay during the two time points.

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60 **Strengths and limitations of this study**

61 • The study captures the exposure risk for Healthcare Workers practicing during a

62 pandemic and the antibody levels due to exposure to SARS-CoV-2 infection.

63 • In this cohort study that included 178 healthcare workers, over a 4-month period

64 following the COVID-19 pandemic, participants had an initial rise in anti-

65 nucleocapsid (N) and anti-spike (S) antibodies, which was followed by decay and

66 stabilization of the titres.

67 • This study is limited by the single institutional data obtained from epicentre of the

68 COVID-19 pandemic and the possibility of recall bias to the responses on the online

69 survey may exist

70 • Another limitation of the study was that for Phase 2 a smaller number of participants

71 followed up due to the Healthcare Workers who volunteered from around the country

72 were transferred back and lost to follow-up.

73

74 Introduction

75 In light of the unprecedented coronavirus disease 2019 (COVID-19) pandemic, understanding
76 the role of the immune system in countering the viral infection is critical not just to design
77 effective antiviral strategies but also to aid us in taking appropriate public health decisions. The
78 early publication of the viral genome led to a rapid development of many nucleic acid based
79 diagnostic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
80 infections. While nucleic acid-based tests are widely employed in the diagnosis of acute
81 (current) SARS-CoV-2 infections, they are often limited in their clinical utility in identifying
82 past infections or assess the level of immunity to SARS-CoV-2 within the communities.
83 Evaluation of antibody responses is the other well-known modality used in a clinical setting
84 that can detect both current, and past infections and is the preferred approach for surveillance
85 to determine the true prevalence of infections. The currently available serological assays for
86 SARS-CoV-2 target either the viral nucleoprotein (N) or the spike surface protein (S) antigens.
87 The S-protein, which contains the receptor binding domain (RBD), binds to host cells via the
88 angiotensin converting-enzyme-2 (ACE2) receptor, followed by membrane fusion^{1,2}. The spike
89 is the target of most neutralizing antibodies³⁻⁵, while the N plays an important role in
90 transcription enhancement and viral assembly⁶. Studies have demonstrated that antibodies
91 against the N and S appeared around the same time - between day 8 and day 14 after the onset
92 of symptoms with antibodies to the N being more sensitive than anti S antibodies for detecting
93 early infection⁷. Neutralizing antibodies confer protective immunity and can be detected in
94 most infected individuals 10-15 days following the onset of COVID-19 symptoms and remain
95 elevated following initial viral clearance⁸⁻¹². There is compelling evidence suggesting that
96 serological assays for anti-S antibodies predict neutralizing activity, in contrast to N based
97 assays^{11,13}.

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The detailed characterization of the dynamics of humoral immune responses to the SARS-CoV-2 viral antigens following infection is still ongoing and early evidences suggest an initial decay of antibody followed by stabilization at a certain level^{11,14-18}. These dynamics are likely driven by an initial expansion of plasmablasts which produce large amounts of antibody but die off quickly followed by a slower decay of antibody titres (the half-life of IgG is approximately three weeks) which then transitions into a steady state level of antibody produced by long-lived plasma cells¹⁹. However, it is currently unknown, if the magnitude of the initial expansion of plasmablast and the associated antibody titres are correlated with the steady state level of serum antibody produced by long-lived plasma cells. This is an important question since steady state antibody levels may provide superior protection from re-infection^{20,21}.

Specifically, there is currently a paucity of information on the kinetics of antibody decay among health care workers (HCW). It is suspected that SARS-CoV-2 infections among HCW are usually asymptomatic or mildly symptomatic and frequently associated with either underreporting of symptoms or heterogenous PCR and/or serologic diagnostics leading to most of them going undetected or unrecognized²². A large cohort study of HCWs in the greater New York City (NYC) area showed a seroprevalence of SARS-CoV-2 antibodies of 13.7%²³. Our own data of anti N antibody screening among HCW at a New York City public hospital in the Bronx following the first “surge” of COVID-19 in May 2020, found that SARS-CoV-2 seroprevalence was at 27%²⁴. Understanding the longitudinal kinetics of SARS-CoV-2 antibody response and the effectiveness of commercial antibody measurement assays is crucial to correctly determine infection rates, sero-prevalence and true sero-reversion rates in both

infected and vaccinated individuals – and to better understand protection associated with sero-positivity.

In this study, we aimed to investigate the longitudinal humoral responses to viral N and the spike and to evaluate their correlation to clinical symptoms and baseline characteristics in our HCW study cohort. Importantly, having access to samples during the initial antibody peak and several months out, we also aimed to determine if initial high antibody levels correlated with high antibody titers at steady state.

Methods

Study setting and population

The study is a prospective cross-sectional cohort study done in two phases after receiving Institutional Review Board approval (IRB # 20-009). The Phase 1 was conducted in May/June, 2020 and the Phase 2 was completed August/September 2020. The cohort included HCWs who worked at the New York City Public Hospital in the South Bronx and were willing to participate in both phases of the study. In the Phase 1 of the study, after informed consent, participants underwent qualitative serology testing (Abbott Architect SARS-CoV-2 IgG Assay, Abbott Park, IL 60064 USA)²⁵ and a nasopharyngeal swab for SARS-CoV-2 (Bio-Reference Laboratories, Inc., Elmwood Park, NJ, USA). They also completed an initial online survey on demographics, symptoms of COVID-19, healthcare/community exposure etc. An extra sample was collected and stored at -80°C for subsequent analysis. These samples were processed using a quantitative enzyme-linked immunosorbent assay (ELISA) that correlates well with virus neutralization, developed by Mount Sinai Health System (MSH ELISA)^{26,27}, to detect RBD and full-length spike (S) reactive antibodies. Participants from Phase 1 who agreed to return

for follow up serology testing (Abbott and MSH ELISA) and completion of a follow-up online survey were part of Phase 2 of the study.

Antibody assays

The Abbott Architect assay uses a qualitative chemiluminescent microparticle immunoassay technology targeting the N antigen of the virus with a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6% (CI 99–99.9%)²⁵. The MSH ELISA consists of an initial ELISA using serum or plasma to detect specific IgG against the RBD of SARS-CoV-2 at a single dilution, followed by quantitative titrations of presumptive positives in a confirmatory ELISA against full length SARS-CoV-2 spike protein (S)²⁸. The positive result from the spike ELISA is reported as antibody at a titre of 1:80 or higher. Test performance assessment revealed that PCR+ samples were 94 % positive and all negative samples returned a negative result for 100% negative agreement²⁹.

Survey

The online survey was accessed by a unique identification number assigned to each participant, blinded to the research team to ensure confidentiality. The survey requested information on age, race/ethnicity, comorbidities, domestic/international travel and healthcare and community exposure details during and prior to both phases. The first phase collected information about symptoms of COVID-19 including their timing and duration in the preceding weeks of the blood draw²⁴. The Phase 2 survey requested information on new comorbidities, persistent COVID-19 symptoms (cough, shortness of breath, anosmia, ageusia, myalgia, nausea, and/or diarrhea), interim testing via antibody and/or reverse transcription polymerase chain reaction (RT -PCR) (if present) and their result (positive/negative), presence of positive SARS-CoV-2 PCR results in the preceding months, interim domestic/international travel and continued use

of personal protective equipment (PPE). The risk of exposure in the healthcare setting and community exposure was determined based on CDC guidelines³⁰.

Statistical analysis

Convenience sampling design was adapted to recruit participants with a goal of 500 participants. Descriptive statistics were used to summarize the baseline characteristics of the cohort and key study outcome variables. Categorical variables were compared by the Chi-squared test, while continuous variables were compared by a Student's t-test. The spike antibody titres were described as geometric means. Correlations were calculated using standard Pearson and Spearman correlation. Multiple linear regression was applied to determine the predictors of log₁₀ rate of decay from Phase 1 to Phase 2 of anti-spike antibodies. A p-value of <0.05 was considered significant. All statistical analyses were performed using SPSS version 27 (IBM, USA).

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

For Phase 1 of our study, 500 healthcare workers underwent both PCR and serology testing. Of these, 137 were positive by for anti-N antibody (Abbott) and 142 were positive by the MSH ELISA. For the second phase 178 participants from the initial cohort consented and underwent evaluation with PCR, antibody assays (Abbott and MSH ELISA) and completed the online follow up survey. The details of patient enrolment are described in **Figure 1**. While 46 of the 178 tested subjects remained positive for the anti-N antibody (Abbott), 70 were positive by the MSH ELISA in the second phase. Anti -spike titres of the 5 subjects in the first phase were close to the cut off for positivity. Twenty-two subjects who were negative for anti-N antibody

in Phase 2 had positive titres of anti-RBD and anti-spike antibodies, though lower than their Phase 1 levels. Among the subjects who participated in the Phase 1 and Phase 2 study, 68 were positive in both phases by the MSH ELISA, 110 were negative in both phases and 2 were positive only in Phase 2 with previously negative results in Phase 1.

The baseline characteristics of study participants who were positive by MSH ELISA in both phases (n=68) and those who were negative in both phases (n=108) are shown in **Table 1**. The mean age of the participants was 44.7±12.4 years, and 63.1% were female. Overall, 30.7% of the HCWs were Latinx, 29.5% were Asian, 16.5% were Black and 17.6% were White. COVID-19 related symptoms were present in 83.8% (57) of the subjects who were positive in both phases, while only 42.6% (46) of the subgroup who had negative antibodies in both phases admitted to symptoms prior to Phase 1. The duration of symptoms prior to Phase 1 was longer among the symptomatic positive group (48.3% for >14 days) in comparison to symptomatic negative group (17.8% for >14 days). The mean duration of symptoms to Phase 1 testing in the symptomatic positive sub cohort was 47.9±16.0 days. Persisting symptoms of COVID-19 were reported in 19 (27.9%) subjects from the cohort with positive antibodies in both phases.

Clinical characteristics and seropositivity to spike protein in both phases

Table 2 describes the characteristics of the symptomatic and asymptomatic subjects who were positive for anti-spike antibody in both phases. Baseline characteristics were comparable between the groups and no difference either in the healthcare or community exposure or in the location of work (ED/Inpatient/intensive care unit, OR etc.) between the two groups was observed. Titres of anti-spike antibodies (geometric mean area under the curve (AUC)) were higher in symptomatic subjects than in asymptomatic positive subjects (6754 AUC vs. 5803 AUC) in Phase 1. However, in the Phase 2 analysis we observed marginally higher titres in the

asymptomatic subgroup compared to the symptomatic subgroup (2383 AUC vs. 2198 AUC). The rate of decay was higher in the symptomatic subgroup (geometric mean 32.96 per day) compared to the asymptomatic (geometric mean 23.42 per day) suggesting delayed antibody/kinetics in the asymptomatic cohort.

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226 **Phase 1 anti-spike antibody titre and clinical correlations**

227 A Pearson's product and Spearman's rank-order correlation was run to assess the relationship
228 between cohort characteristics including age, gender, comorbidities, number of symptoms of
229 COVID-19, healthcare exposure and Phase 1 anti-spike titres in our cohort (**Figure 2**). One
230 hundred-forty-three subjects with a positive test in Phase 1 were included in the analysis.
231 Scatter plot analysis showed a monotonic relationship between the variables. A statistically
232 significant weak positive correlation was observed between age and Phase 1 anti-spike
233 antibody titres ($R=0.269$, $p<0.005$). Moderate positive correlation was present between
234 presence of fever ($R=0.319$, $p<0.005$), number of symptoms ($R=0.310$, $p<0.005$) and days of
235 symptoms ($R=0.434$, $p<0.005$) and anti-spike antibody titre; and weak positive correlation was
236 observed with upper respiratory symptoms ($R=0.278$, $p<0.005$) and gastrointestinal (GI)
237 symptoms ($R=0.204$, $p<0.05$) with anti-spike antibody titres.

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239 **Correlation of rate of decay of anti-spike antibody titres from Phase 1 to Phase 2 and** 240 **clinical characteristics**

241 Results of Pearson correlation to assess the relationship between cohort characteristics
242 including Phase 1 anti-spike antibody titres, age, gender, comorbidities, symptoms of COVID-
243 19, number of symptoms of COVID-19, healthcare exposure and decay of anti-spike titres
244 between the two phases in our cohort is shown in **Figure 3**. A strong positive statistically
245 significant correlation was observed between Phase 1 titers and decay of anti-spike antibody

titres between the two phases ($R=0.898$, $p<0.000$). Medium positive correlation was observed between presence of fever ($R=0.428$, $p<0.001$), GI symptoms ($R=0.340$, $p<0.011$), number of symptoms ($R=0.357$, $p<0.007$), duration of symptoms ($R=0.469$, $p<0.000$) with decay of anti-spike antibody titres between the two phases respectively.

A pairwise comparison was performed between rate of decay of anti-spike antibody titres and patient characteristics (**Figure 4**). Rate of decay by gender was comparable (male; 30.73 AUC/day vs. female; 34.68 AUC/day, $p=0.413$). Asian (86.0 AUC/day) race showed higher rate of decay compared with White (7.2 AUC/day) and Black (19.61 AUC/day) individuals; while Latinx (47.28 AUC/day) race had higher rate of decay compared with White (7.2 AUC/day) individuals. Subjects with fever had a higher rate of decay than those who did not report fever (53.08 AUC/day vs. 16.14 AUC/day, $p=0.002$). Similarly subjects with GI symptoms had a higher rate of decay than those without (55.81 AUC/day vs. 21.94 AUC/day, $p=0.019$). Subjects with symptoms restricted to less than seven days demonstrated a lower decay rate compared with symptomatic subjects over 7-14 days (13.60 AUC/day vs. 36.12 AUC/day, $p=0.046$) and when compared with symptomatic subjects with more than 14 days (13.60 AUC/day vs. 59.72 AUC/day, $p=0.001$). This finding was statistically significant. No difference was found when degree of exposure (High/Moderate: 28.18 AUC/day vs. Mild: 34.78 AUC/day, $p=0.395$) or job role (physician: 29.57 AUC/day vs. nurse: 53.59 AUC/day vs. Other: 26.83 AUC/day; $p=0.361$) was compared to rate of decay.

Predictors of rate of decay from Phase 1 to Phase 2 of anti-spike antibodies

Multiple linear regression analysis to predict the rate of decay with respect to age, Bacillus Calmette Guerin vaccination, number of symptoms, and Phase 1 (log10) anti-spike antibody titres is shown in **Table 3**. On the basis of a linear regression model that included the

participants age, history of BCG vaccination, total number of COVID-19 symptoms and the Phase 1 concentration of log 10 spike antibody titres, the estimated change (decay) was 23.6 AUC/day when age was centred at median (42.6 years), there was positive history of BCG vaccination, the total number of COVID-19 symptoms were centred at a median of 4, and the geometric mean of the log₁₀ spike antibody titre was 3.78.

Discussion

With the COVID-19 pandemic showing no signs of abating, healthcare workers at the epicentre are at risk of infection due to occupational exposure as well as community exposure. Sero-surveillance is the foundation for determining the scale and rate of exposures. With a multitude of serological assays getting emergency use approval from FDA, interpretation of the results of these assays and their clinical significance remains challenging. It is critical to understand the timing of the antibody response for acute interpretation. Confidence in analytical specificity of the assay is a critical requirement in measurement of the specific antibody responses. Recent studies have confirmed that anti spike titres especially anti-RBD titres can serve as surrogates for virus neutralization^{31,32}. The Abbott SARS-CoV-2 IgG assay that targets antibodies to the nucleoprotein has a reported specificity and sensitivity of greater than 99% at 14 days or more following symptom onset and these measurements are not indicative or correlated to virus neutralization titres³³. In comparison, the MSH ELISA targets the full-length S protein including RBD, a major target for neutralizing antibodies and has demonstrated excellent correlation to virus neutralization^{11,26}. Longitudinal measurements of antibody levels have revealed that anti-N and anti S IgG antibodies continue to increase until the third week post symptoms and an approach that combines the detection of both of these antibodies would precisely detect almost 100% of all infectious exposures³⁴. In our study, the mean number of days after

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10 299 Longitudinal blood sampling among HCWs working at a public hospital in the epicentre of
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12 300 the pandemic in NYC allowed for analysis of kinetics of anti-S and anti-N antibody
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14 301 responses. At two months after the first surge of infections, anti-N antibodies were detected
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16 302 in 27% and anti-S antibodies in 28% of participating HCWs. After an interval of four months,
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18 303 it is not surprising to note that among the participants who returned, 26% remained positive
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20 304 for anti N antibodies, while 31% of the previously anti-N antibody positive subjects tested
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22 305 negative in phase 2. On the other hand, a similar analysis of the anti-S antibodies levels,
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24 306 confirmed that all the previously positive retested subjects continued to remain positive,
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31 309 COVID-19 related symptoms were significantly associated with positive anti-spike antibodies
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33 310 in both phases, with a similar association with longer duration (>14 days) of symptoms.
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35 311 Previous studies have demonstrated a lower level of IgG response among patients without
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37 312 symptoms or with mild symptoms compared to those with severe and critical disease^{35,36}. A
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39 313 comparison of symptomatic versus asymptomatic subjects who tested positive for anti-spike
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41 314 antibodies in both phases, confirmed that the rate of decay of anti-spike antibody titres were
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43 315 faster in the symptomatic cohort than the asymptomatic subjects, which was seen also in the
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45 316 anti-N antibody kinetics. However, we observed a faster decay in this group with a lower titre
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47 317 of anti-spike antibodies in Phase 2 compared to the asymptomatic cohort (though the difference
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49 318 was not statistically significant). This could additionally be supported by the finding of fever
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51 319 and GI symptoms contributing to faster decay. Similar results of decreasing neutralizing
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53 320 antibody titre in symptomatic than asymptomatic patients were observed by Choe *et al.*³⁷
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322 Positive correlations for age, presence of fever, upper respiratory symptoms, GI symptoms,

323 total number and duration of symptoms was observed with increased levels of anti-spike titres

324 at Phase 1. Similar results of neutralizing antibody titres were also observed by

325 Boonyaratanakornkit *et al.* wherein they showed higher levels of neutralizing antibody titres

326 were significantly associated with male gender, older adults, higher disease severity and shorter

327 interval from recovery³⁸. Based on a linear regression model with age centred at median (42.6

328 years), positive history of BCG vaccination, the total number of COVID-19 symptoms centred

329 at a median of 4, and the geometric mean of the log10 anti-spike antibody titre at 3.78, we

330 observed that the rate of decay of these antibody titres was 23.6 AUC/day. Evaluation of other

331 characteristics with rate of decay between Phase 1 and Phase 2 showed a faster reduction in

332 titres in Asian participants and in those with fever and GI symptoms. A slower decrease was

333 noted among patients with shorter duration (<7 days) of symptoms, with no other significant

334 correlation noted with any other baseline demographics or clinical characteristics.

335 As described above, higher antibody titers are associated with a larger number of symptoms,

336 longer duration of symptoms and – as described by others as well – disease severity in general.

337 We also found that higher initial antibody titers were associated with faster antibody decay

338 during the two time points. Initial antibody responses are driven by short lived plasmablasts,

339 which decay after a few days after producing massive amounts of antibody. IgG has a relatively

340 long half-life of approximately three weeks, but decay is inevitable since the plasmablasts

341 initially producing it disappear. Usually, titers then drop until they reach relatively stable levels

342 of antibody which are maintained by long-lived plasma cells in the bone marrow¹⁹. The two

343 time points described in this study represent the initial peak response and likely the stable level

344 after the initial decay. We found that individuals with higher initial titers had a faster decay

345 rate during the observation period meaning the difference between peak and stable, long-lived

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antibody levels were larger. This indicates that there is likely no direct correlation between the magnitude of the initial expansion of plasmablasts and the number of long-lived plasma cells that migrate to the bone marrow.

Our study has the following limitations: First being a single center study with a small convenience sampling that included a smaller number of participants in Phase 2 of the study. Following the pandemic, the HCWs who volunteered from around the country were transferred back and lost to follow-up, which did decrease the overall sample size, but the rates of positive and negative results remained proportional. Second, the likelihood of a recall bias in the participant’s responses on the online survey may exist. Lastly, as a cross-sectional seroprevalence study the findings can underestimate rates of prior infections based on timing of the testing given that antibodies are only transiently detectable following infection.

In conclusion, findings from this study are similar to other studies that have reported that higher magnitude of anti-spike titres may correlate with protection against reinfection, in spite of the observed decay in the antibody levels^{20,21}. Nevertheless, further studies to evaluate the longevity of immunity, especially in context of widespread administration of spike-based vaccine among HCWs would be important in predicting herd immunity to COVID-19 infections.

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Deceased: Author Bo Yu MD has deceased and will always be remembered.

Contributions: V.M., M.A.S. and U.V. designed the study. J.M.C., M.A.S., B.Y., V.P.G. analysed the data. E.V. and M.G. assisted with participant follow-up and coordination with the assistance of A.P., U.V., V.M., and M.A.S. The Mount Sinai Health System team, J.M.C. and F.K., performed the measurements for anti-Spike and Anti-RBD antibodies. V.M., U.V. and A.P. were responsible for the clinical care of the research participants and supervised the day-to-day operation and coordination of the study by M.K., V.D., M.A.S., B.Y., V.P.G., M.G., E.V., and M.G. V.M. and F.K wrote the manuscript and is the guarantor of this work and has full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, with the assistance of J.C.Q., M.A.S., B.Y., V.P.G., and M.G. All authors critically revised the draft and approved the final manuscript.

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Competing Interests: F.K. is listed as a co-inventor on a patent application filed by The Icahn School of Medicine at Mount Sinai relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines. Mount Sinai has spun out a company, Kantaro, to market serological tests for

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SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020), and is currently consulting for Seqirus and Avimex. F.K.'s Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not required

Ethics approval: The study protocol was approved by the Institutional Review Board approval (IRB # 20-009, Lincoln Medical Center, Office of the Institutional Review Board approved as per 45 CFR 46 & 21 CFR50,56 under a full board committee and gave its approval on 4/28/2020). All participants provided written informed consent for the use of their data.

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417 References

- 418 1. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on
419 virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun.*
420 2020;11(1):1620.
- 421 2. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Velesler D. Structure,
422 Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell.*
423 2020;181(2):281-292.
- 424 3. Premkumar L, Segovia-Chumbez B, Jadi R, et al. The receptor binding domain of the
425 viral spike protein is an immunodominant and highly specific target of antibodies in
426 SARS-CoV-2 patients. *Sci Immunol.* 2020;5(48):eabc8413.
- 427 4. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular
428 Immunity in COVID-19 Convalescent Individuals. *Immunity.* 2020;52(6):971-977
- 429 5. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody
430 responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19
431 patients. *Sci Immunol.* 2020; 5(52):eabe0367.
- 432 6. Cong Y, Ulasli M, Schepers H, et al. Nucleocapsid Protein Recruitment to Replication-
433 Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. *J Virol.*
434 2020;94(4):e01925-19.
- 435 7. Burbelo PD, Riedo FX, Morishima C, et al. Detection of Nucleocapsid Antibody to
436 SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients.
437 medRxiv [Preprint]. 2020;2020.04.20.20071423
- 438 8. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK. Antibody responses to SARS-
439 CoV-2 in patients with COVID-19. *Nat Med.* 2020;26(6):845-848.
- 440 9. İnandiklioğlu N, Akkoc T. Immune Responses to SARS-CoV, MERS-CoV and SARS-
441 CoV-2. *Adv Exp Med Biol.* 2020;1288:5-12.
- 442 10. Chaudhuri S, Thiruvengadam R, Chattopadhyay S, et al. Comparative evaluation of
443 SARS-CoV-2 IgG assays in India. *J Clin Virol.* 2020;131:104609.
- 444 11. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2
445 infection persist for months. *Science.* 2020;370(6521):1227-1230.
- 446 12. Crawford KHD, Dingens AS, Eguia R, et al. Dynamics of neutralizing antibody titers
447 in the months after SARS-CoV-2 infection. *J Infect Dis.* 2020;jiaa618.
- 448 13. Luchsinger LL, Ransegnola BP, Jin DK, et al. Serological Assays Estimate Highly
449 Variable SARS-CoV-2 Neutralizing Antibody Activity in Recovered COVID-19
450 Patients. *J Clin Microbiol.* 2020;58(12)
- 451 14. Iyer AS, Jones FK, Nodoushani A, et al. Dynamics and significance of the antibody
452 response to SARS-CoV-2 infection. medRxiv. 2020;2020.07.18.20155374.
- 453 15. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses to
454 SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol.* 2020;5(52):
455 eabe5511.
- 456 16. Wu F, Wang A, Liu m, et al. Neutralizing antibody responses to SARS-CoV-2 in a
457 COVID-19 recovered patient cohort and their implications. medRxiv,
458 2020;2020.03.30.20047365.
- 459 17. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of
460 neutralizing antibody responses in the three months following SARS-CoV-2 infection
461 in humans. *Nat Microbiol.* 2020;5(12):1598-1607.
- 462 18. Grandjean L, Saso A, Torres A, et al. Humoral Response Dynamics Following Infection
463 with SARS-CoV-2. medRxiv, 2020;2020.07.16.20155663.
- 464 19. Ellebedy A, Turner J, Kim W, et al. SARS-CoV-2 infection induces long-lived bone

1
2
3 465 marrow plasma cells in humans. *Res Sq [Preprint]*. 2020;rs.3.rs-132821.
4 466 20. Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody Status and Incidence of SARS-
5 467 CoV-2 Infection in Health Care Workers. *N Engl J Med*. 2021;384(6):533-540.
6 468 21. Hall V, Foulkes S, Charlett A, et al. Do antibody positive healthcare workers have lower
7 469 SARS-CoV-2 infection rates than antibody negative healthcare workers? Large multi-
8 470 centre prospective cohort study (the SIREN study), England: June to November 2020.
9 471 *medRxiv*, 2021;2021.01.13.21249642.
10 472 22. Self WH, Tenforde MW, Stubblefield WB, et al. Seroprevalence of SARS-CoV-2
11 473 Among Frontline Health Care Personnel in a Multistate Hospital Network. 13
12 474 Academic Medical Centers, April-June 2020. *MMWR Morb Mortal Wkly Rep*.
13 475 2020;69(35):1221-1226.
14 476 23. Moscola J, Sembajwe G, Jarrett M, et al. Prevalence of SARS-CoV-2 Antibodies in
15 477 Health Care Personnel in the New York City Area. *JAMA*. 2020;324(9):893-895.
16 478 24. Venugopal U, Jilani N, Rabah S, et al. SARS-CoV-2 seroprevalence among health care
17 479 workers in a New York City hospital: A cross-sectional analysis during the COVID-19
18 480 pandemic. *Int J Infect Dis*. 2021;102:63-69
19 481 25. H Hamilton F, Muir P, Attwood M, et al. Kinetics and performance of the Abbott
20 482 architect SARS-CoV-2 IgG antibody assay. *J Infect*. 2020;81(6):e7-e9.
21 483 26. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2
22 484 seroconversion in humans. *Nat Med*. 2020;26(7):1033-1036.
23 485 27. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in
24 486 Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test
25 487 Setup. *Curr Protoc Microbiol*. 2020;57(1):e100.
26 488 28. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2
27 489 seroconversion in humans. *Nat Med*. 2020;26(7):1033-1036.
28 490 29. Stadlbauer D, Tan J, Jiang K, et al. Repeated cross-sectional sero-monitoring of SARS-
29 491 CoV-2 in New York City. *Nature*. 2021;590(7844):146-150.
30 492 30. Garcia M, Lipskiy N, Tyson J, Watkins R, Esser ES, Kinley T. Centers for Disease
31 493 Control and Prevention 2019 novel coronavirus disease (COVID-19) information
32 494 management: addressing national health-care and public health needs for standardized
33 495 data definitions and codified vocabulary for data exchange. *J Am Med Inform Assoc*.
34 496 2020;27(9):1476-1487
35 497 31. Therrien C, Serhir B, Bélanger-Collard M, et al. Multicenter Evaluation of the Clinical
36 498 Performance and the Neutralizing Antibody Activity Prediction Properties of ten high
37 499 throughput serological assays used in Clinical Laboratories. *J Clin Microbiol*.
38 500 2020;JCM.02511-20.
39 501 32. Muecksch F, Wise H, Batchelor B, et al. Longitudinal analysis of clinical serology
40 502 assay performance and neutralising antibody levels in COVID19 convalescents.
41 503 *medRxiv*, 2020;2020.08.05.20169128.
42 504 33. Rosadas C, Randell P, Khan M, McClure MO, Tedder RS. Testing for responses to the
43 505 wrong SARS-CoV-2 antigen? *Lancet*. 2020;396(10252):e23.
44 506 34. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses
45 507 in COVID-19 patients. *Emerg Microbes Infect*. 2020;9(1):940-948.
46 508 35. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of
47 509 asymptomatic SARS-CoV-2 infections. *Nat Med*. 2020; 26(8):1200-1204.
48 510 36. Wang Y, Zhang L, Sang L, et al. Kinetics of viral load and antibody response in relation
49 511 to COVID-19 severity. *J Clin Invest*. 2020;130(10):5235-5244.
50 512 37. Choe PG, Kang CK, Suh HJ, et al. Waning Antibody Responses in Asymptomatic and
51 513 Symptomatic SARS-CoV-2 Infection. *Emerg Infect Dis*. 2021;27(1):327-329.
52 514

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2
3 515 38. Boonyaratanakornkit J, Morishima C, Selke S, et al. Clinical, laboratory, and temporal
4 516 predictors of neutralizing antibodies to SARS-CoV-2 after COVID-19. medRxiv. 2020;
5 517 2020.10.06.20207472.
6 518
7
8
9 519
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11 520
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Table 1: Broad characteristics among health care workers assessed for antibody reactivity to spike SARS-CoV-2 protein in Phase 1 and Phase 2

	Overall †	Spike ELISA (AUC) positive in both phases	Negative Reactivity to spike (AUC) in both phases	p value
	N=176	n=68	n=108	
Age, years	44.7+12.4	42.9+11.9	45.8+12.7	0.099
Female, Gender	111 (63.1%)	40 (58.8%)	71 (65.7%)	0.467
Race				0.666
Latinx	54 (30.7%)	21 (30.9%)	33 (30.6%)	
Asian	52 (29.5%)	18 (26.5%)	34 (31.5%)	
Black	29 (16.5%)	15 (22.1%)	14 (13.0%)	
White	31 (17.6%)	10 (14.7%)	21 (19.4%)	
Other	10 (5.7%)	4 (5.9%)	6 (5.9%)	
Comorbidities	54 (30.7%)	25 (36.8%)	29 (26.9%)	0.214
BCG vaccine received in childhood	87 (49.4%)	35 (51.5%)	52 (48.1%)	0.902
COVID-19 related symptoms prior to Phase 1	103 (58.5%)	57 (83.8%)	46 (42.6%)	<.001
Duration of symptoms				<.001
<7 days	48 (46.6%)	18 (31.0%)	30 (66.7%)	
7-14 days	19 (18.4%)	12 (20.7%)	7 (15.6%)	
>14 days	36 (35.0%)	28 (48.3%)	8 (17.8%)	
Time from symptom to positive result, days	45.7+19.9	47.9+16.0	42.9+24.1	0.062
RT-PCR positive result for SARS-CoV-2 prior to Phase 1	51 (29.0%)	49 (72.1%)	2 (1.9%)	<.001
RT-PCR positive result for SARS-CoV-2 during Phase 1	14 (8.0%)	13 (19.1%)	1 (0.9%)	<.001
Persisting symptoms from COVID-19	25 (14.2%)	19 (27.9%)	6 (5.6%)	<.001
Nature of work				0.306
Physicians	81 (46.0%)	29 (42.6%)	52 (51.5%)	
Nurses	29 (16.5%)	15 (22.1%)	14 (13.0%)	
Others	64 (36.4%)	24 (35.3%)	40 (39.6%)	
Hospital areas worked in:				
Emergency department/Inpatient units	118 (67.0%)	50 (73.5%)	68 (63.0%)	0.141
Ambulatory care/Clinics	72 (40.9%)	27 (39.7%)	45 (41.7%)	0.631
Administration/Non-clinical care areas	24 (13.6%)	9 (13.2%)	15 (13.9%)	0.867
Community exposure	47 (26.7%)	19 (27.9%)	28 (25.9%)	0.591

Household exposure	39 (22.2%)	17 (25.0%)	22 (20.4%)	0.343
PPE use at work	173 (98.3%)	67 (98.5%)	106 (98.1%)	0.226
Use of facemask outside of the hospital	158 (89.8%)	58 (85.3%)	100 (92.6%)	0.062

Continuous variables are expressed as mean (SD), categorical variables as n (%).
BCG, Bacillus Calmette–Guérin vaccine; PPE, personal protective equipment; RT-PCR, reverse transcription polymerase chain reaction.

† Demographic data is missing for 2 participants from the overall cohort.

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Table 2: Broad characteristics among health care workers with positive antibody reactivity to SARS-CoV-2 spike in both phases

	Overall	Asymptomatic for SARS-CoV-2 infection	Symptomatic for SARS- CoV-2 infection	<i>p</i> <i>value</i>
	n=68	n=11	n=57	
Age, Mean (±SD)	42.9 (±1.45)	44.5 (±3.8)	42.6 (±1.6)	0.557
Female, n (%)	40 (58.8%)	6 (54.5%)	34 (40.4%)	0.502
Race				0.753
Latinx	21 (30.9%)	3 (27.3%)	18 (31.6%)	
Asian	18 (26.5%)	3 (27.3%)	18 (26.3%)	
Black	15 (22.1%)	3 (27.3%)	12 (21.1%)	
White	10 (14.7%)	2 (18.2%)	8 (14.0%)	
Other	4 (5.8%)	0 (0%)	4 (7.0%)	
Comorbidities				
Hypertension	13 (19.1%)	2 (18.2%)	11 (19.3%)	0.650
Diabetes	6 (8.8%)	0 (0%)	6 (10.5%)	0.332
COPD and asthma	13 (19.1%)	1 (9.1%)	12 (21.1%)	0.326
Number of symptoms, median (IQR)	-	-	4.0 (2.0-5.0)	
Length of symptoms				
<7 days	-	-	19 (33.3%)	
7-14 days	-	-	12 (21.1%)	
>14 days	-	-	26 (45.6%)	
Degree of HCW exposure				0.492
High and Moderate	16 (23.5%)	2 (18.2%)	14 (24.6%)	
Minor	52 (76.5%)	9 (81.8%)	43 (75.4%)	
Community exposure	19 (27.9%)	3 (27.3%)	16 (28.1%)	0.635
Household exposure	17 (25.4%)	3 (27.3%)	14 (24.6%)	0.557

Use of facemask outside of hospital	58 (85.3%)	9 (81.8%)	49 (86.0%)	0.722
Principal means of transportation				0.663
Public	33 (48.5%)	6 (54.5%)	27 (47.7%)	
Private	35 (51.5%)	5 (45.5%)	30 (52.6%)	
Nature of work				0.502
Physician	29 (42.6%)	4 (36.4%)	25 (43.9%)	
Nurse	15 (22.1%)	2 (18.2%)	13 (22.8%)	
Other	24 (35.3%)	5 (45.5%)	19 (33.3%)	
Hospital areas work in:				0.288
Emergency department/inpatient units	32 (47.1%)	6 (54.5%)	26 (45.6%)	
Ambulatory care/clinics	9 (13.2%)	2 (18.2%)	7 (12.3%)	
Inpatient and outpatient setting	18 (26.5%)	3 (27.3%)	15 (26.3%)	
Administration/nonclinical care areas	9 (13.2%)	0 (0%)	9 (15.8%)	
Anti-spike reactivity (AUC)				
Reactivity in phase 1, G-Mean (IQR)	6590 (5165-8410)	5803 (2825-11920)	6754 (5177-8812)	0.647
Days from symptoms to first test, Mean (\pm SD)	-	-	47.7 (\pm 1.9)	
Reactivity in phase 2, G-Mean (IQR)	2226 (1824-2718)	2382 (1494-3797)	2198 (1753-2755)	0.980
Days from symptoms to second test			174.5 (\pm 4.1)	
Rate of decay, G-Mean (IQR)	31.14 (22.11-43.87)	23.42 (8.45-64.93)	32.96 (22.73-47.82)	0.382

Continuous variables are expressed as mean (SD) or interquartile range (IQR), categorical variables as n (%).

AUC, area under the curve; COPD, Chronic obstructive pulmonary disease; HCW, health care worker

Table 3: Multiple linear regression analysis of rate of decay for anti-spike antibodies between Phase 1 and Phase 2

Rate of decay (log10)	<i>B</i>	95.0% CI for <i>B</i>		<i>SE B</i>	β	<i>R</i> ²	$\blacktriangle R^2$
		LL	UL				
Model						0.83	0.82
Constant	-3.203**	-3.647	-2.759	.222			
Age (per 10-year change)	.014	-.005	.007	.002	.030		
BCG vaccination	.131**	.030	.310	.046	.121		
Number of symptoms	.013	-.029	.060	.012	.050		
ELISA reactivity (Log10)	1.159**	1.050	1.419	.059	.916		

B: Unstandardized regression coefficient; CI: confidence interval; LL: lower limit; UL: upper limit; *SE B*: standard error of the coefficient; β : standardized coefficient; *R*²: coefficient of determination; $\blacktriangle R^2$: adjusted *R*².

***P* < 0.05

BCG, Bacillus Calmette–Guérin vaccine

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531 Figure 1: Flow Chart of patient enrollment, follow up and analysis

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533 Figure 2: Simple correlation analysis of HCW with positive reactivity for anti- spike antibody

534 in Phase 1 with baseline characteristics and symptoms

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536 Figure 3: Simple correlation analysis of rate of decay of anti-spike antibodies between both

537 phases with baseline characteristics and symptoms

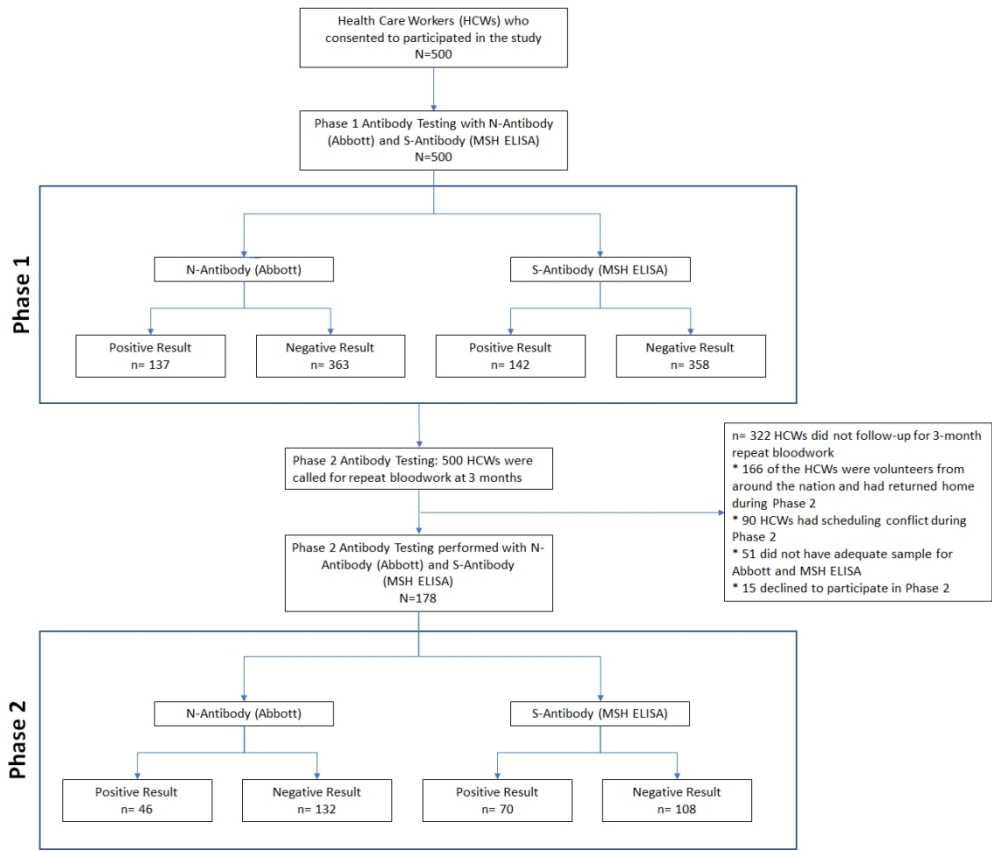
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539 Figure 4: Paired comparison between rate of decay of anti-spike antibody titres and patient

540 characteristics

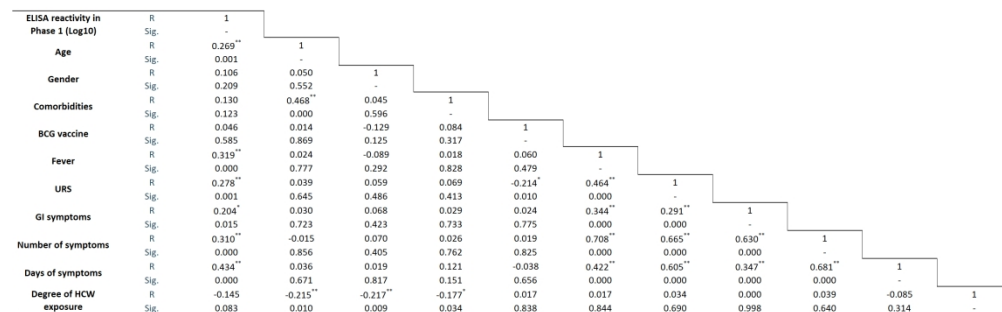
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Flow Chart of patient enrollment, follow up and analysis

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		ELISA reactivity in Phase 1 (Log10)	Age	Gender	Comorbidities	BCG vaccine	Fever	URS	GI symptoms	Number of symptoms	Days of symptoms	Degree of HCW exposure
ELISA reactivity in Phase 1 (Log10)	R	1										
	Sig.											
Age	R	0.269**	1									
	Sig.	0.001										
Gender	R	0.106	0.050	1								
	Sig.	0.209	0.552									
Comorbidities	R	0.130	0.468**	0.045	1							
	Sig.	0.123	0.000	0.596								
BCG vaccine	R	0.046	0.014	-0.129	0.084	1						
	Sig.	0.585	0.869	0.125	0.317							
Fever	R	0.319**	0.024	-0.089	0.018	0.060	1					
	Sig.	0.000	0.777	0.292	0.828	0.479						
URS	R	0.278**	0.039	0.059	0.069	-0.214*	0.464**	1				
	Sig.	0.001	0.645	0.486	0.413	0.010	0.000					
GI symptoms	R	0.204*	0.030	0.068	0.029	0.024	0.344**	0.291**	1			
	Sig.	0.015	0.723	0.423	0.733	0.775	0.000	0.000				
Number of symptoms	R	0.310**	-0.015	0.070	0.026	0.019	0.708**	0.665**	0.630**	1		
	Sig.	0.000	0.856	0.405	0.762	0.825	0.000	0.000	0.000			
Days of symptoms	R	0.434**	0.036	0.019	0.121	-0.038	0.422**	0.605**	0.347**	0.681**	1	
	Sig.	0.000	0.671	0.817	0.151	0.656	0.000	0.000	0.000	0.000		
Degree of HCW exposure	R	-0.145	-0.215**	-0.217**	-0.177*	0.017	0.017	0.034	0.000	0.039	-0.085	1
	Sig.	0.083	0.010	0.009	0.034	0.838	0.844	0.690	0.998	0.640	0.314	

BCG, Bacillus Calmette-Guérin; URS, upper respiratory symptoms; GI, Gastrointestinal symptoms (nausea, vomit and diarrhoea); HCW, health care worker.
 **, Correlation is significant at the 0.01 level, *, Correlation is significant at the 0.05 level.

Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms

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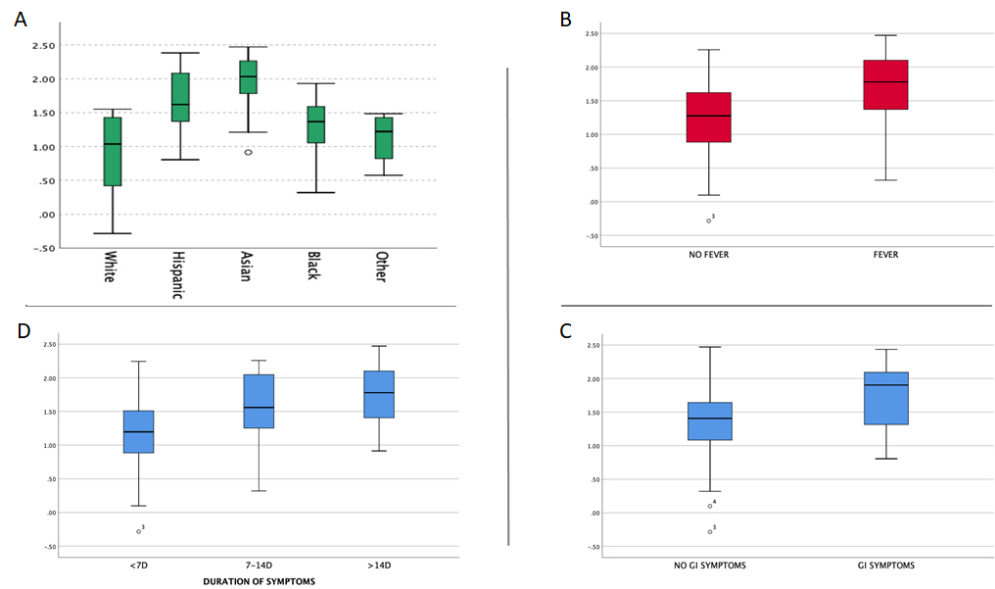
Rate of decay (Log10)	R	1									
	sig.	-									
ELISA reactivity (Log10)	R	0.898**	1								
	sig.	0	-								
Age	R	0.176	0.182	1							
	sig.	0.198	0.184	-							
Gender	R	0.044	0.094	0.001	1						
	sig.	0.751	0.493	0.994	-						
Comorbidity	R	0.193	0.156	0.514**	-0.092	1					
	sig.	0.158	0.257	0.000	0.502	-					
Fever	R	0.428**	0.463**	-0.090	-0.090	-0.122	1				
	sig.	0.001	0.000	0.515	0.512	0.374	-				
GI symptoms	R	0.340*	0.289*	-0.020	-0.072	-0.163	0.269*	1			
	sig.	0.011	0.033	0.885	0.603	0.234	0.047	-			
Number of symptoms	R	0.357**	0.360**	-0.256	0.008	-0.193	0.640**	0.555**	1		
	sig.	0.007	0.007	0.059	0.954	0.158	0.000	0.000	-		
Duration of symptoms	R	0.469**	0.469**	-0.049	0.123	0.142	0.204	0.207	0.430**	1	
	sig.	0.000	0.000	0.723	0.370	0.300	0.136	0.129	0.001	-	
Degree of HCW exposure	R	0.067	0.106	0.154	0.266*	0.202	-0.136	-0.075	-0.184	0.133	1
	sig.	0.625	0.439	0.261	0.050	0.140	0.321	0.586	0.180	0.334	-
		Rate of decay (Log10)	ELISA reactivity (Log10)	Age	Gender	Comorbidity	Fever	GI symptoms	Number of symptoms	Duration of symptoms	Degree of HCW exposure

BCG: Bacillus Calmette-Guérin; GI: Gastrointestinal symptoms (nausea, vomit and diarrhea); HCW: health care worker.

** Correlation is significant at the 0.01 level, * Correlation is significant at the 0.05 level

Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms

686x269mm (96 x 96 DPI)



Paired comparison between rate of decay of anti-spike antibody titres and patient characteristics

270x161mm (96 x 96 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the STROBE cohortreporting guidelines, and cite them as:

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			Page Number
Reporting Item			
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	7
Methods			

Study design	#4	Present key elements of study design early in the paper	7
Setting	#5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.	7
Eligibility criteria	#6b	For matched studies, give matching criteria and number of exposed and unexposed	7
Variables	#7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7
Data sources / measurement	#8	For each variable of interest give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group. Give information separately for for exposed and unexposed groups if applicable.	8
Bias	#9	Describe any efforts to address potential sources of bias	16
Study size	#10	Explain how the study size was arrived at	9
Quantitative variables	#11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	7-9
Statistical methods	#12a	Describe all statistical methods, including those used to control for confounding	
Statistical methods	#12b	Describe any methods used to examine subgroups and interactions	9
Statistical methods	#12c	Explain how missing data were addressed	9
Statistical methods	#12d	If applicable, explain how loss to follow-up was addressed	9
Statistical methods	#12e	Describe any sensitivity analyses	

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3	Results			
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6	Participants	#13a	Report numbers of individuals at each stage of study—eg	9
7			numbers potentially eligible, examined for eligibility, confirmed	
8			eligible, included in the study, completing follow-up, and	
9			analysed. Give information separately for for exposed and	
10			unexposed groups if applicable.	
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14	Participants	#13b	Give reasons for non-participation at each stage	30
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16	Participants	#13c	Consider use of a flow diagram	
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18	30			
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21	Descriptive data	#14a	Give characteristics of study participants (eg demographic,	9,23-24
22			clinical, social) and information on exposures and potential	
23			confounders. Give information separately for exposed and	
24			unexposed groups if applicable.	
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28	Descriptive data	#14b	Indicate number of participants with missing data for each	
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34	Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	
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39	Outcome data	#15	Report numbers of outcome events or summary measures	
40			over time. Give information separately for exposed and	
41			unexposed groups if applicable.	
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46	Main results	#16a	Give unadjusted estimates and, if applicable, confounder-	10-11
47			adjusted estimates and their precision (eg, 95% confidence	
48			interval). Make clear which confounders were adjusted for and	
49			why they were included	
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53	Main results	#16b	Report category boundaries when continuous variables were	10
54			categorized	
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57	Main results	#16c	If relevant, consider translating estimates of relative risk into	
58			absolute risk for a meaningful time period	
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Other analyses	#17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-13
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13-14
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	16
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	15-16
Generalisability	#21	Discuss the generalisability (external validity) of the study results	16-17
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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BMJ Open

Longitudinal Humoral Antibody Response to SARs-CoV2 Infection among Health Care Workers in a New York City Hospital

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Longitudinal Humoral Antibody Response to SARS-CoV2 infection among Health Care Workers in a New York City Hospital

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Abstract

Objective

Dynamics of humoral immune responses to SARS-CoV-2 antigens following infection suggests an initial decay of antibody followed by subsequent stabilization. We aim to understand the longitudinal humoral responses to SARS-CoV-2 nucleocapsid (N) protein and spike (S) protein and to evaluate their correlation to clinical symptoms among healthcare workers (HCW).

Design

A prospective cross-sectional cohort study.

Setting

This study was conducted in New York City Public Hospital in the South Bronx, New York.

Participants

Healthcare Workers participated in Phase 1 (N=500) and Phase 2 (N=178) of our study and underwent both PCR and serology testing, in addition to online survey. Analysis was performed on the 178 participants that presented for both phases of the study.

Primary outcome measure

Data from both phases over four months was collected on HCW that underwent serial qualitative serology testing for anti-N antibody, quantitative MSH-ELISA to detect Receptor Binding Domain and full-length S reactive antibodies to measure the decay rate and stabilization of the titres for SARS-CoV-2 infection.

Results

Anti-N antibody positivity was 27% and anti-S positivity was 28% in Phase 1. In Phase 1 anti-S titres were higher in symptomatic (6754(5177-8812) than in asymptomatic positive subjects (5803(2825-11920). Marginally higher titers (2382(1494-3797) were seen in asymptomatic compared to the symptomatic positive subgroup (2198 (1753-2755) in Phase

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2. A positive correlation was noted between age ($R=0.269$, $p<0.005$), number ($R=0.310$, $p<0.005$) and duration of symptoms ($R=0.434$, $p<0.005$), and Phase 1 anti-S antibody titre. A strong correlation ($R=0.898$, $p<0.000$). was observed between Phase 1 titers and decay of anti-S antibody titres between the two phases. Significant correlation with rate of decay was also noted with fever ($R=0.428$, $p<0.001$), GI symptoms ($R=0.340$, $p<0.011$), and total number ($R=0.357$, $p<0.007$) and duration of COVID-19 symptoms ($R=0.469$, $p<0.000$).

Conclusions

Higher initial anti-S antibody titres were associated with larger number and longer duration of symptoms as well as faster decay during the two time points.

Strengths and limitations of this study

- The strength of our study is the longitudinal design with serial sampling to determine humoral response to SARS-CoV-2 infection from consenting Health Care Workers during the pandemic.
- This study collected serial detailed characteristics of symptomatic and asymptomatic Health Care Workers to correlate with durability and decay of humoral response.
- This study is limited by the single institutional data obtained from epicentre of the COVID-19 pandemic and the possibility of recall bias to the responses on the online survey may exist
- Our cohort for Phase 2 was smaller than Phase 1, due to discontinuation of volunteer healthcare workers from the surge period.”

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71 **Introduction**

72 In light of the unprecedented coronavirus disease 2019 (COVID-19) pandemic, understanding
73 the role of the immune system in countering the viral infection is critical not just to design
74 effective antiviral strategies but also to aid us in taking appropriate public health decisions. The
75 early publication of the viral genome led to a rapid development of many nucleic acid based
76 diagnostic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
77 infections. While nucleic acid-based tests are widely employed in the diagnosis of acute
78 (current) SARS-CoV-2 infections, they are often limited in their clinical utility in identifying
79 past infections or assess the level of immunity to SARS-CoV-2 within the communities.
80 Evaluation of antibody responses is the other well-known modality used in a clinical setting
81 that can detect both current, and past infections and is the preferred approach for surveillance
82 to determine the true prevalence of infections. The currently available serological assays for
83 SARS-CoV-2 target either the viral nucleoprotein (N) or the spike surface protein (S) antigens.
84 The S-protein, which contains the receptor binding domain (RBD), binds to host cells via the
85 angiotensin converting-enzyme-2 (ACE2) receptor, followed by membrane fusion^{1,2}. The spike
86 is the target of most neutralizing antibodies ³⁻⁵, while the N plays an important role in
87 transcription enhancement and viral assembly ⁶. Studies have demonstrated that antibodies
88 against the N and S appeared around the same time - between day 8 and day 14 after the onset
89 of symptoms with antibodies to the N being more sensitive than anti S antibodies for detecting
90 early infection⁷. Neutralizing antibodies confer protective immunity and can be detected in
91 most infected individuals 10-15 days following the onset of COVID-19 symptoms and remain
92 elevated following initial viral clearance ⁸⁻¹². There is compelling evidence suggesting that
93 serological assays for anti-S antibodies predict neutralizing activity, in contrast to N based
94 assays^{11,13}.

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96 The detailed characterization of the dynamics of humoral immune responses to the SARS-
97 CoV-2 viral antigens following infection is still ongoing and early evidences suggest an initial
98 decay of antibody followed by stabilization at a certain level^{11,14-18}. These dynamics are likely
99 driven by an initial expansion of plasmablasts which produce large amounts of antibody but
100 die off quickly followed by a slower decay of antibody titres (the half-life of IgG is
101 approximately three weeks) which then transitions into a steady state level of antibody
102 produced by long-lived plasma cells¹⁹. However, it is currently unknown, if the magnitude of
103 the initial expansion of plasmablast and the associated antibody titres are correlated with the
104 steady state level of serum antibody produced by long-lived plasma cells. This is an important
105 question since steady state antibody levels may provide superior protection from re-
106 infection^{20,21}.

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108 Specifically, there is currently a paucity of information on the kinetics of antibody decay among
109 health care workers (HCW). It is suspected that SARS-CoV-2 infections among HCW are
110 usually asymptomatic or mildly symptomatic and frequently associated with either
111 underreporting of symptoms or heterogenous PCR and/or serologic diagnostics leading to most
112 of them going undetected or unrecognized²². A large cohort study of HCWs in the greater New
113 York City (NYC) area showed a seroprevalence of SARS-CoV-2 antibodies of 13.7%²³. Our
114 own data of anti N antibody screening among HCW at a New York City public hospital in the
115 Bronx following the first “surge” of COVID-19 in May 2020, found that SARS-CoV-2
116 seroprevalence was at 27%²⁴. Understanding the longitudinal kinetics of SARS-CoV-2
117 antibody response and the effectiveness of commercial antibody measurement assays is crucial
118 to correctly determine infection rates, sero-prevalence and true sero-reversion rates in both

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3 119 infected and vaccinated individuals – and to better understand protection associated with sero-
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5 120 positivity.
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10 122 In this study, we aimed to investigate the longitudinal humoral responses to viral N and the
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12 123 spike and to evaluate their correlation to clinical symptoms and baseline characteristics in our
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14 124 HCW study cohort. Importantly, having access to samples during the initial antibody peak and
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17 125 several months out, we also aimed to determine if initial high antibody levels correlated with
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19 126 high antibody titers at steady state.
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24 128 **Methods**

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26 129 **Study setting and population**

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28 130 The study is a prospective cross-sectional cohort study done in two phases after receiving
29
30 131 Institutional Review Board approval (IRB # 20-009). The Phase 1 was conducted in May/June,
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32 132 2020 and the Phase 2 was completed August/September 2020. The cohort included HCWs who
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34 133 worked at the New York City Public Hospital in the South Bronx and were willing to participate
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36 134 in both phases of the study. Information about the study was disseminated among health care
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38 135 workers via intranet informative bulletins, research staff approaching on duty staff and handing
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40 136 our study flyers and introducing the study in multiple department meetings. During Phase 2,
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42 137 the HCWs that had participated for Phase 1 were called individually to schedule an
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44 138 appointment with research staff for blood-work and survey completion.
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51 140 In the Phase 1 of the study, after informed consent, participants underwent qualitative serology
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53 141 testing (Abbott Architect SARS-CoV-2 IgG Assay, Abbott Park, IL 60064 USA)²⁵ and a
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55 142 nasopharyngeal swab for SARS-CoV-2 (Bio-Reference Laboratories, Inc., Elmwood Park, NJ,
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57 143 USA). They also completed an initial online survey on demographics, symptoms of COVID-
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19 including duration, and healthcare/community exposure. An extra sample was collected and stored at -80°C for subsequent analysis. These samples were processed using a quantitative enzyme-linked immunosorbent assay (ELISA) that correlates well with virus neutralization, developed by Mount Sinai Health System (MSH ELISA)^{26,27}, to detect RBD and full-length spike (S) reactive antibodies; and in Phase 2 for follow up serology testing was also performed with Abbott and MSH ELISA.

Antibody assays

The Abbott Architect assay uses a qualitative chemiluminescent microparticle immunoassay technology targeting the N antigen of the virus with a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6% (CI 99–99.9%)²⁵. The MSH ELISA consists of an initial ELISA using serum or plasma to detect specific IgG against the RBD of SARS-CoV-2 at a single dilution, followed by quantitative titrations of presumptive positives in a confirmatory ELISA against full length SARS-CoV-2 spike protein (S)²⁸. The positive result from the spike ELISA is reported as antibody at a titre of 1:80 or higher. Test performance assessment revealed that PCR+ samples were 94 % positive and all negative samples returned a negative result for 100% negative agreement²⁹.

Survey

The online survey was accessed by a unique identification number assigned to each participant, blinded to the research team to ensure confidentiality. The survey was designed for the purpose of this study to capture demographics and current medical history, with emphasis on COVID-19 infection exposure, symptomology, duration of symptoms and persistence of symptoms from exposure prior to Phase 1 (period prior to May 2020). Survey was piloted amongst staff prior to use in this study. The survey requested information on age, race/ethnicity,

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3 169 comorbidities, domestic/international travel and healthcare and community exposure details
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5 170 during and prior to both phases. The first phase collected information about symptoms of
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7 171 COVID-19 including their timing and duration in the preceding weeks of the blood draw²⁴.
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10 172 The Phase 2 survey requested information on new comorbidities, persistent COVID-19
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12 173 symptoms (cough, shortness of breath, anosmia, ageusia, myalgia, nausea, and/or diarrhea),
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14 174 interim testing via antibody and/or reverse transcription polymerase chain reaction (RT -PCR)
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16 175 (if present) and their result (positive/negative), presence of positive SARS-CoV-2 PCR results
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18 176 in the preceding months, interim domestic/international travel and continued use of personal
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20 177 protective equipment (PPE). The risk of exposure in the healthcare setting and community
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22 178 exposure was determined based on CDC guidelines³⁰.
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28 180 **Statistical analysis**

30 181 Convenience sampling design was adapted to recruit participants with a goal of 500
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32 182 participants. Descriptive statistics were used to summarize the baseline characteristics of the
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34 183 cohort and key study outcome variables. Categorical variables were compared by the Chi-
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36 184 squared test, while continuous variables were compared by a Student's t-test. The spike
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38 185 antibody titres were described as geometric means. Correlations were calculated using standard
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40 186 Pearson and Spearman correlation. Multiple linear regression was applied to determine the
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42 187 predictors of log10 rate of decay from Phase 1 to Phase 2 of anti-spike antibodies. A p-value
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44 188 of <0.05 was considered significant. All statistical analyses were performed using SPSS
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46 189 version 27 (IBM, USA).
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53 191 **Patient and Public Involvement**

55 192 Patients or the public were not involved in the design, or conduct, or reporting, or
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57 193 dissemination plans of our research.
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Results

For Phase 1 of our study, 500 healthcare workers underwent both PCR and serology testing. Of these, 137 were positive by for anti-N antibody (Abbott) and 142 were positive by the MSH ELISA. For the second phase 178 participants from the initial cohort consented and underwent evaluation with PCR, antibody assays (Abbott and MSH ELISA) and completed the online follow up survey. The follow-up occurred 133 ± 21 days between Phase 1 and Phase 2. The details of patient enrolment are described in **Figure 1**. While 46 of the 178 tested subjects remained positive for the anti-N antibody (Abbott), 70 were positive by the MSH ELISA in the second phase. Anti -spike titres of the 5 subjects in the first phase were close to the cut off for positivity. Twenty-two subjects who were negative for anti-N antibody in Phase 2 had positive titres of anti-RBD and anti-spike antibodies, though lower than their Phase 1 levels. Among the subjects who participated in the Phase 1 and Phase 2 study, 68 were positive in both phases by the MSH ELISA, 110 were negative in both phases and 2 were positive only in Phase 2 with previously negative results in Phase 1.

The baseline characteristics of study participants who were positive by MSH ELISA in both phases (n=68) and those who were negative in both phases (n=108) are shown in **Table 1**. The mean age of the participants was 44.7 ± 12.4 years, and 63.1% were female. Overall, 30.7% of the HCWs were Latinx, 29.5% were Asian, 16.5% were Black and 17.6% were White. COVID-19 related symptoms were present in 83.8% (57) of the subjects who were positive in both phases, while only 42.6% (46) of the subgroup who had negative antibodies in both phases admitted to symptoms prior to Phase 1. The duration of symptoms prior to Phase 1 was longer among the symptomatic positive group (48.3% for >14 days) in comparison to symptomatic negative group (17.8% for >14 days). The mean duration of symptoms to Phase 1 testing in the

symptomatic positive sub cohort was 47.9 ± 16.0 days. Persisting symptoms of COVID-19 were reported in 19 (27.9%) subjects from the cohort with positive antibodies in both phases.

Clinical characteristics and seropositivity to spike protein in both phases

Table 2 describes the characteristics of the symptomatic and asymptomatic subjects who were positive for anti-spike antibody in both phases. Baseline characteristics were comparable between the groups and no difference either in the healthcare or community exposure or in the location of work (ED/Inpatient/intensive care unit, OR etc.) between the two groups was observed. Titres of anti-spike antibodies (geometric mean area under the curve (AUC)) were higher in symptomatic subjects than in asymptomatic positive subjects (6754 AUC vs. 5803 AUC) in Phase 1. However, in the Phase 2 analysis we observed marginally higher titres in the asymptomatic subgroup compared to the symptomatic subgroup (2383 AUC vs. 2198 AUC). Figure 2 illustrates the symptomatic and asymptomatic antibody levels of anti-spike antibodies. The rate of decay was higher in the symptomatic subgroup (geometric mean 32.96 per day) compared to the asymptomatic (geometric mean 23.42 per day) suggesting delayed antibody/kinetics in the asymptomatic cohort.

Phase 1 anti-spike antibody titre and clinical correlations

A Pearson's product and Spearman's rank-order correlation was run to assess the relationship between cohort characteristics including age, gender, comorbidities, number of symptoms of COVID-19, healthcare exposure and Phase 1 anti-spike titres in our cohort (**Figure 3**). One hundred-forty-three subjects with a positive test in Phase 1 were included in the analysis. Scatter plot analysis showed a monotonic relationship between the variables. A statistically significant weak positive correlation was observed between age and Phase 1 anti-spike antibody titres ($R=0.269$, $p<0.005$). Moderate positive correlation was present between

presence of fever ($R=0.319$, $p<0.005$), number of symptoms ($R=0.310$, $p<0.005$) and days of symptoms ($R=0.434$, $p<0.005$) and anti-spike antibody titre; and weak positive correlation was observed with upper respiratory symptoms ($R=0.278$, $p<0.005$) and gastrointestinal (GI) symptoms ($R=0.204$, $p<0.05$) with anti-spike antibody titres.

Correlation of rate of decay of anti-spike antibody titres from Phase 1 to Phase 2 and clinical characteristics

Results of Pearson correlation to assess the relationship between cohort characteristics including Phase 1 anti-spike antibody titres, age, gender, comorbidities, symptoms of COVID-19, number of symptoms of COVID-19, healthcare exposure and decay of anti-spike titres between the two phases in our cohort is shown in **Figure 4**. A strong positive statistically significant correlation was observed between Phase 1 titers and decay of anti-spike antibody titres between the two phases ($R=0.898$, $p<0.000$). Medium positive correlation was observed between presence of fever ($R=0.428$, $p<0.001$), GI symptoms ($R=0.340$, $p<0.011$), number of symptoms ($R=0.357$, $p<0.007$), duration of symptoms ($R=0.469$, $p<0.000$) with decay of anti-spike antibody titres between the two phases respectively.

A pairwise comparison was performed between rate of decay of anti-spike antibody titres and patient characteristics (**Figure 5**). Rate of decay by gender was comparable (male; 30.73 AUC/day vs. female; 34.68 AUC/day, $p=0.413$). Asian (86.0 AUC/day) race showed higher rate of decay compared with White (7.2 AUC/day) and Black (19.61 AUC/day) individuals; while Latinx (47.28 AUC/day) race had higher rate of decay compared with White (7.2 AUC/day) individuals. Subjects with fever had a higher rate of decay than those who did not report fever (53.08 AUC/day vs. 16.14 AUC/day, $p=0.002$). Similarly subjects with GI

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3 268 symptoms had a higher rate of decay than those without (55.81 AUC/day vs.21.94 AUC/day,
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5 269 $p=0.019$). Subjects with symptoms restricted to less than seven days demonstrated a lower
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8 270 decay rate compared with symptomatic subjects over 7-14 days (13.60 AUC/day vs. 36.12
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10 271 AUC/day, $p=0.046$) and when compared with symptomatic subjects with more than 14 days
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12 272 (13.60 AUC/day vs. 59.72 AUC/day, $p=0.001$). This finding was statistically significant. No
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14 273 difference was found when degree of exposure (High/Moderate: 28.18 AUC/day vs. Mild:
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16 274 34.78 AUC/day, $p=0.395$) or job role (physician: 29.57 AUC/day vs. nurse: 53.59 AUC/day
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18 275 vs. Other: 26.83 AUC/day; $p=0.361$) was compared to rate of decay.
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24 277 **Predictors of rate of decay from Phase 1 to Phase 2 of anti-spike antibodies**

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26 278 Multiple linear regression analysis to predict the rate of decay with respect to age, Bacillus
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28 279 Calmette Guerin vaccination, number of symptoms, and Phase 1 (\log_{10}) anti-spike antibody
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30 280 titres is shown in **Table 3**. On the basis of a linear regression model that included the
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32 281 participants age, history of BCG vaccination, total number of COVID-19 symptoms and the
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34 282 Phase 1 concentration of log 10 spike antibody titres, the estimated change (decay) was 23.6
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36 283 AUC/day when age was centred at median (42.6 years), there was positive history of BCG
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38 284 vaccination, the total number of COVID-19 symptoms were centred at a median of 4, and the
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40 285 geometric mean of the \log_{10} spike antibody titre was 3.78.
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47 287 **Discussion**

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49 288 With the COVID-19 pandemic showing no signs of abating, healthcare workers at the
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51 289 epicentre are at risk of infection due to occupational exposure as well as community
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53 290 exposure. Sero-surveillance is the foundation for determining the scale and rate of exposures.
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55 291 With a multitude of serological assays getting emergency use approval from FDA,
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57 292 interpretation of the results of these assays and their clinical significance remains
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challenging. It is critical to understand the timing of the antibody response for acute interpretation. Confidence in analytical specificity of the assay is a critical requirement in measurement of the specific antibody responses. Recent studies have confirmed that anti spike titres especially anti-RBD titres can serve as surrogates for virus neutralization^{31,32}. The Abbott SARS-CoV-2 IgG assay that targets antibodies to the nucleoprotein has a reported specificity and sensitivity of greater than 99% at 14 days or more following symptom onset and these measurements are not indicative or correlated to virus neutralization titres³³. In comparison, the MSH ELISA targets the full-length S protein including RBD, a major target for neutralizing antibodies and has demonstrated excellent correlation to virus neutralization^{11,26}. Longitudinal measurements of antibody levels have revealed that anti-N and anti S IgG antibodies continue to increase until the third week post symptoms and an approach that combines the detection of both of these antibodies would precisely detect almost 100% of all infectious exposures³⁴. In our study, the mean number of days after symptoms to testing in Phase 1 was 47 days suggesting a higher likelihood of accuracy of the utilized assay.

Longitudinal blood sampling among HCWs working at a public hospital in the epicentre of the pandemic in NYC allowed for analysis of kinetics of anti-S and anti-N antibody responses. At two months after the first surge of infections, anti-N antibodies were detected in 27% and anti-S antibodies in 28% of participating HCWs. After an interval of four months, it is not surprising to note that among the participants who returned, 26% remained positive for anti N antibodies, while 31% of the previously anti-N antibody positive subjects tested negative in phase 2. On the other hand, a similar analysis of the anti-S antibodies levels, confirmed that all the previously positive retested subjects continued to remain positive, albeit with lower titres. That being said, we acknowledge that the decline of N antibodies in

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our cohort could be due to the Abbott assay being less sensitive to describe the dynamics of N antibodies over time compared to other assays, like Roche, Siemens and Diasorin. Muecksch et al. demonstrated in their longitudinal analysis of clinical serology assay performance among COVID-19 convalescents, that there is a difference in diagnostic performance among various serologic assays³².

COVID-19 related symptoms were significantly associated with positive anti-spike antibodies in both phases, with a similar association with longer duration (>14 days) of symptoms. Previous studies have demonstrated a lower level of IgG response among patients without symptoms or with mild symptoms compared to those with severe and critical disease^{35,36}. A comparison of symptomatic versus asymptomatic subjects who tested positive for anti-spike antibodies in both phases, confirmed that the rate of decay of anti-spike antibody titres were faster in the symptomatic cohort than the asymptomatic subjects, which was seen also in the anti-N antibody kinetics. However, we observed a faster decay in this group with a lower titre of anti-spike antibodies in Phase 2 compared to the asymptomatic cohort (though the difference was not statistically significant). This could additionally be supported by the finding of fever and GI symptoms contributing to faster decay. Similar results of decreasing neutralizing antibody titre in symptomatic than asymptomatic patients were observed by Choe *et al.*³⁷

Positive correlations for age, presence of fever, upper respiratory symptoms, GI symptoms, total number and duration of symptoms was observed with increased levels of anti-spike titres at Phase 1. Similar results of neutralizing antibody titres were also observed by Boonyaratanakornkit *et al.* wherein they showed higher levels of neutralizing antibody titres were significantly associated with male gender, older adults, higher disease severity and shorter interval from recovery³⁸. Based on a linear regression model with age centred at median (42.6

years), positive history of BCG vaccination, the total number of COVID-19 symptoms centred at a median of 4, and the geometric mean of the log₁₀ anti-spike antibody titre at 3.78, we observed that the rate of decay of these antibody titres was 23.6 AUC/day. Evaluation of other characteristics with rate of decay between Phase 1 and Phase 2 showed a faster reduction in titres in Asian participants and in those with fever and GI symptoms. A slower decrease was noted among patients with shorter duration (<7 days) of symptoms, with no other significant correlation noted with any other baseline demographics or clinical characteristics.

As described above, higher antibody titers are associated with a larger number of symptoms, longer duration of symptoms and – as described by others as well – disease severity in general. We also found that higher initial antibody titers were associated with faster antibody decay during the two time points. Initial antibody responses are driven by short lived plasmablasts, which decay after a few days after producing massive amounts of antibody. IgG has a relatively long half-life of approximately three weeks, but decay is inevitable since the plasmablasts initially producing it disappear. Usually, titers then drop until they reach relatively stable levels of antibody which are maintained by long-lived plasma cells in the bone marrow¹⁹. The two time points described in this study represent the initial peak response and likely the stable level after the initial decay. We found that individuals with higher initial titers had a faster decay rate during the observation period meaning the difference between peak and stable, long-lived antibody levels were larger. This indicates that there is likely no direct correlation between the magnitude of the initial expansion of plasmablasts and the number of long-lived plasma cells that migrate to the bone marrow. It is critical to recognize that steady state antibody titers are similar between the symptomatic and asymptomatic subgroups, suggesting that mid-term humoral protection might be similar after infection regardless of disease severity.

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Our study has the following limitations: First being a single center study with a small convenience sampling that included a smaller number of participants in Phase 2 of the study. Following the pandemic, the HCWs who volunteered from around the country were transferred back and lost to follow-up, which did decrease the overall sample size, but the rates of positive and negative results remained proportional. Second, the likelihood of a recall bias in the participant’s responses on the online survey may exist. Lastly, as a cross-sectional seroprevalence study the findings can underestimate rates of prior infections based on timing of the testing given that antibodies are only transiently detectable following infection.

In conclusion, findings from this study are similar to other studies that have reported that higher magnitude of anti-spike titres may correlate with protection against reinfection, in spite of the observed decay in the antibody levels^{20,21}. Nevertheless, further studies to evaluate the longevity of immunity, especially in context of widespread administration of spike-based vaccine among HCWs would be important in predicting herd immunity to COVID-19 infections.

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399
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401 analysed the data. E.V. and M.G. assisted with participant follow-up and coordination with
402 the assistance of A.P., U.V., V.M., and M.A.S. The Mount Sinai Health System team, J.M.C.
403 and F.K., performed the measurements for anti-Spike and Anti-RBD antibodies. V.M., U.V.
404 and A.P. were responsible for the clinical care of the research participants and supervised the
405 day-to-day operation and coordination of the study by M.K., V.D., M.A.S., B.Y., V.P.G.,
406 M.G., E.V., and M.G. V.M. and F.K wrote the manuscript and are the guarantors of this work
407 and have full access to all data in the study and take responsibility for the integrity of the data
408 and the accuracy of the data analysis, with the assistance of J.C.Q., M.A.S., B.Y., V.P.G., and
409 M.G. All authors critically revised the draft and approved the final manuscript.

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412
413 **Competing Interests:** F.K. is listed as a co-inventor on a patent application filed by The Icahn
414 School of Medicine at Mount Sinai relating to SARS-CoV-2 serological assays and NDV-based
415 SARS-CoV-2 vaccines. Mount Sinai has spun out a company, Kantaro, to market serological tests for
416 SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020), and is currently consulting for

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417 Seqirus and Avimex. F.K.’s Krammer laboratory is also collaborating with Pfizer on animal models
418 of SARS-CoV-2.
419 All other authors report no potential conflicts. All authors have submitted the ICMJE Form
420 for Disclosure of Potential Conflicts of Interest.

422 **Patient and public involvement:** Patients and/or the public were not involved in the design,
423 or conduct, or reporting, or dissemination plans of this research.

425 **Patient consent for publication:** Not required

427 **Ethics approval:** The study protocol was approved by the Institutional Review Board
428 approval (IRB # 20-009, Lincoln Medical Center, Office of the Institutional Review Board
429 approved as per 45 CFR 46 & 21 CFR50,56 under a full board committee and gave its
430 approval on 4/28/2020). All participants provided written informed consent for the use of
431 their data.

433 Provenance and peer review Not commissioned; externally peer reviewed.

435 **Data availability statement:** Data are available on request from the corresponding author.
436 All data relevant to the study has been included in the article.

References

1. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun.* 2020;11(1):1620.
2. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell.* 2020;181(2):281-292.
3. Premkumar L, Segovia-Chumbez B, Jadi R, et al. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci Immunol.* 2020;5(48):eabc8413.
4. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. *Immunity.* 2020;52(6):971-977
5. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Sci Immunol.* 2020; 5(52):eabe0367.
6. Cong Y, Ulasli M, Schepers H, et al. Nucleocapsid Protein Recruitment to Replication-Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. *J Virol.* 2020;94(4):e01925-19.
7. Burbelo PD, Riedo FX, Morishima C, et al. Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients. *medRxiv [Preprint].* 2020;2020.04.20.20071423
8. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med.* 2020;26(6):845-848.
9. İnandiklioğlu N, Akkoc T. Immune Responses to SARS-CoV, MERS-CoV and SARS-CoV-2. *Adv Exp Med Biol.* 2020;1288:5-12.
10. Chaudhuri S, Thiruvengadam R, Chattopadhyay S, et al. Comparative evaluation of SARS-CoV-2 IgG assays in India. *J Clin Virol.* 2020;131:104609.
11. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science.* 2020;370(6521):1227-1230.
12. Crawford KHD, Dingens AS, Eguia R, et al. Dynamics of neutralizing antibody titers in the months after SARS-CoV-2 infection. *J Infect Dis.* 2020;jiaa618.
13. Luchsinger LL, Ransegnola BP, Jin DK, et al. Serological Assays Estimate Highly Variable SARS-CoV-2 Neutralizing Antibody Activity in Recovered COVID-19 Patients. *J Clin Microbiol.* 2020;58(12)
14. Iyer AS, Jones FK, Nodoushani A, et al. Dynamics and significance of the antibody response to SARS-CoV-2 infection. *medRxiv.* 2020;2020.07.18.20155374.
15. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci Immunol.* 2020;5(52):eabe5511.
16. Wu F, Wang A, Liu m, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. *medRxiv,* 2020;2020.03.30.20047365.
17. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat Microbiol.* 2020;5(12):1598-1607.
18. Grandjean L, Saso A, Torres A, et al. Humoral Response Dynamics Following Infection with SARS-CoV-2. *medRxiv,* 2020;2020.07.16.20155663.
19. Ellebedy A, Turner J, Kim W, et al. SARS-CoV-2 infection induces long-lived bone

- marrow plasma cells in humans. *Res Sq [Preprint]*. 2020;rs.3.rs-132821.
20. Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody Status and Incidence of SARS-CoV-2 Infection in Health Care Workers. *N Engl J Med*. 2021;384(6):533-540.
 21. Hall V, Foulkes S, Charlett A, et al. Do antibody positive healthcare workers have lower SARS-CoV-2 infection rates than antibody negative healthcare workers? Large multi-centre prospective cohort study (the SIREN study), England: June to November 2020. *medRxiv*, 2021;2021.01.13.21249642.
 22. Self WH, Tenforde MW, Stubblefield WB, et al. Seroprevalence of SARS-CoV-2 Among Frontline Health Care Personnel in a Multistate Hospital Network. *13 Academic Medical Centers*, April-June 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69(35):1221-1226.
 23. Moscola J, Sembajwe G, Jarrett M, et al. Prevalence of SARS-CoV-2 Antibodies in Health Care Personnel in the New York City Area. *JAMA*. 2020;324(9):893-895.
 24. Venugopal U, Jilani N, Rabah S, et al. SARS-CoV-2 seroprevalence among health care workers in a New York City hospital: A cross-sectional analysis during the COVID-19 pandemic. *Int J Infect Dis*. 2021;102:63-69
 25. H Hamilton F, Muir P, Attwood M, et al. Kinetics and performance of the Abbott architect SARS-CoV-2 IgG antibody assay. *J Infect*. 2020;81(6):e7-e9.
 26. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*. 2020;26(7):1033-1036.
 27. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol*. 2020;57(1):e100.
 28. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med*. 2020;26(7):1033-1036.
 29. Stadlbauer D, Tan J, Jiang K, et al. Repeated cross-sectional sero-monitoring of SARS-CoV-2 in New York City. *Nature*. 2021;590(7844):146-150.
 30. Garcia M, Lipskiy N, Tyson J, Watkins R, Esser ES, Kinley T. Centers for Disease Control and Prevention 2019 novel coronavirus disease (COVID-19) information management: addressing national health-care and public health needs for standardized data definitions and codified vocabulary for data exchange. *J Am Med Inform Assoc*. 2020;27(9):1476-1487
 31. Therrien C, Serhir B, Bélanger-Collard M, et al. Multicenter Evaluation of the Clinical Performance and the Neutralizing Antibody Activity Prediction Properties of ten high throughput serological assays used in Clinical Laboratories. *J Clin Microbiol*. 2020;JCM.02511-20.
 32. Muecksch F, Wise H, Batchelor B, et al. Longitudinal Serological Analysis and Neutralizing Antibody Levels in Coronavirus Disease 2019 Convalescent Patients. *J Infect Dis*. 2021;223(3):389-398.
 33. Rosadas C, Randell P, Khan M, McClure MO, Tedder RS. Testing for responses to the wrong SARS-CoV-2 antigen? *Lancet*. 2020;396(10252):e23.
 34. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. *Emerg Microbes Infect*. 2020;9(1):940-948.
 35. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med*. 2020; 26(8):1200-1204.
 36. Wang Y, Zhang L, Sang L, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. *J Clin Invest*. 2020;130(10):5235-5244.
 37. Choe PG, Kang CK, Suh HJ, et al. Waning Antibody Responses in Asymptomatic and Symptomatic SARS-CoV-2 Infection. *Emerg Infect Dis*. 2021;27(1):327-329.

- 1
2
3 535 38. Boonyaratanakornkit J, Morishima C, Selke S, et al. Clinical, laboratory, and temporal
4 536 predictors of neutralizing antibodies to SARS-CoV-2 after COVID-19. medRxiv. 2020;
5 537 2020.10.06.20207472.
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Table 1: Broad characteristics among health care workers assessed for antibody reactivity to spike SARS-CoV-2 protein in Phase 1 and Phase 2

	Overall †	Spike ELISA (AUC) positive in both phases	Negative Reactivity to spike (AUC) in both phases	p value
	N=176	n=68	n=108	
Age, years	44.7+12.4	42.9+11.9	45.8+12.7	0.099
Female, Gender	111 (63.1%)	40 (58.8%)	71 (65.7%)	0.467
Race				0.666
Latinx	54 (30.7%)	21 (30.9%)	33 (30.6%)	
Asian	52 (29.5%)	18 (26.5%)	34 (31.5%)	
Black	29 (16.5%)	15 (22.1%)	14 (13.0%)	
White	31 (17.6%)	10 (14.7%)	21 (19.4%)	
Other	10 (5.7%)	4 (5.9%)	6 (5.9%)	
Comorbidities	54 (30.7%)	25 (36.8%)	29 (26.9%)	0.214
BCG vaccine received in childhood	87 (49.4%)	35 (51.5%)	52 (48.1%)	0.902
COVID-19 related symptoms prior to Phase 1	103 (58.5%)	57 (83.8%)	46 (42.6%)	<.001
Duration of symptoms				<.001
<7 days	48 (46.6%)	18 (31.0%)	30 (66.7%)	
7-14 days	19 (18.4%)	12 (20.7%)	7 (15.6%)	
>14 days	36 (35.0%)	28 (48.3%)	8 (17.8%)	
Time from symptom to positive result, days	45.7+19.9	47.9+16.0	42.9+24.1	0.062
RT-PCR positive result for SARS-CoV-2 prior to Phase 1	51 (29.0%)	49 (72.1%)	2 (1.9%)	<.001
RT-PCR positive result for SARS-CoV-2 during Phase 1	14 (8.0%)	13 (19.1%)	1 (0.9%)	<.001
Persisting symptoms from COVID-19	25 (14.2%)	19 (27.9%)	6 (5.6%)	<.001
Nature of work				0.306
Physicians	81 (46.0%)	29 (42.6%)	52 (51.5%)	
Nurses	29 (16.5%)	15 (22.1%)	14 (13.0%)	
Others	64 (36.4%)	24 (35.3%)	40 (39.6%)	
Hospital areas worked in:				
Emergency department/Inpatient units	118 (67.0%)	50 (73.5%)	68 (63.0%)	0.141
Ambulatory care/Clinics	72 (40.9%)	27 (39.7%)	45 (41.7%)	0.631
Administration/Non-clinical care areas	24 (13.6%)	9 (13.2%)	15 (13.9%)	0.867
Community exposure	47 (26.7%)	19 (27.9%)	28 (25.9%)	0.591

Household exposure	39 (22.2%)	17 (25.0%)	22 (20.4%)	0.343
PPE use at work	173 (98.3%)	67 (98.5%)	106 (98.1%)	0.226
Use of facemask outside of the hospital	158 (89.8%)	58 (85.3%)	100 (92.6%)	0.062

Continuous variables are expressed as mean (SD), categorical variables as n (%).
BCG, Bacillus Calmette–Guérin vaccine; PPE, personal protective equipment; RT-PCR, reverse transcription polymerase chain reaction.

† Demographic data is missing for 2 participants from the overall cohort.

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Table 2: Broad characteristics among health care workers with positive antibody reactivity to SARS-CoV-2 spike in both phases

	Overall	Asymptomatic for SARS-CoV-2 infection	Symptomatic for SARS- CoV-2 infection	<i>p</i> <i>value</i>
	n=68	n=11	n=57	
Age, Mean (±SD)	42.9 (±1.45)	44.5 (±3.8)	42.6 (±1.6)	0.557
Female, n (%)	40 (58.8%)	6 (54.5%)	34 (40.4%)	0.502
Race				0.753
Latinx	21 (30.9%)	3 (27.3%)	18 (31.6%)	
Asian	18 (26.5%)	3 (27.3%)	18 (26.3%)	
Black	15 (22.1%)	3 (27.3%)	12 (21.1%)	
White	10 (14.7%)	2 (18.2%)	8 (14.0%)	
Other	4 (5.8%)	0 (0%)	4 (7.0%)	
Comorbidities				
Hypertension	13 (19.1%)	2 (18.2%)	11 (19.3%)	0.650
Diabetes	6 (8.8%)	0 (0%)	6 (10.5%)	0.332
COPD and asthma	13 (19.1%)	1 (9.1%)	12 (21.1%)	0.326
Number of symptoms, median (IQR)	-	-	4.0 (2.0-5.0)	
Length of symptoms				
<7 days	-	-	19 (33.3%)	
7-14 days	-	-	12 (21.1%)	
>14 days	-	-	26 (45.6%)	
Degree of HCW exposure				0.492
High and Moderate	16 (23.5%)	2 (18.2%)	14 (24.6%)	
Minor	52 (76.5%)	9 (81.8%)	43 (75.4%)	
Community exposure	19 (27.9%)	3 (27.3%)	16 (28.1%)	0.635
Household exposure	17 (25.4%)	3 (27.3%)	14 (24.6%)	0.557
Use of facemask outside of hospital	58 (85.3%)	9 (81.8%)	49 (86.0%)	0.722

Principal means of transportation	0.663			
Public	33 (48.5%)	6 (54.5%)	27 (47.7%)	
Private	35 (51.5%)	5 (45.5%)	30 (52.6%)	
Nature of work	0.502			
Physician	29 (42.6%)	4 (36.4%)	25 (43.9%)	
Nurse	15 (22.1%)	2 (18.2%)	13 (22.8%)	
Other	24 (35.3%)	5 (45.5%)	19 (33.3%)	
Hospital areas work in:	0.288			
Emergency department/inpatient units	32 (47.1%)	6 (54.5%)	26 (45.6%)	
Ambulatory care/clinics	9 (13.2%)	2 (18.2%)	7 (12.3%)	
Inpatient and outpatient setting	18 (26.5%)	3 (27.3%)	15 (26.3%)	
Administration/nonclinical care areas	9 (13.2%)	0 (0%)	9 (15.8%)	
Anti-spike reactivity (AUC)				
Reactivity in phase 1, G-Mean (IQR)	6590 (5165-8410)	5803 (2825-11920)	6754 (5177-8812)	0.647
Days from symptoms to first test, Mean (\pm SD)	-	-	47.7 (\pm 1.9)	
Reactivity in phase 2, G-Mean (IQR)	2226 (1824-2718)	2382 (1494-3797)	2198 (1753-2755)	0.980
Days from symptoms to second test			174.5 (\pm 4.1)	
Rate of decay, G-Mean (IQR)	31.14 (22.11-43.87)	23.42 (8.45-64.93)	32.96 (22.73-47.82)	0.382

Continuous variables are expressed as mean (SD) or interquartile range (IQR), categorical variables as n (%).

AUC, area under the curve; COPD, Chronic obstructive pulmonary disease; HCW, health care worker

Table 3: Multiple linear regression analysis of rate of decay for anti-spike antibodies between Phase 1 and Phase 2

Rate of decay (log10)	<i>B</i>	95.0% CI for <i>B</i>		<i>SE B</i>	β	<i>R</i> ²	$\blacktriangle R^2$
		LL	UL				
Model						0.83	0.82
Constant	-3.203**	-3.647	-2.759	.222			
Age (per 10-year change)	.014	-.005	.007	.002	.030		
BCG vaccination	.131**	.030	.310	.046	.121		
Number of symptoms	.013	-.029	.060	.012	.050		
ELISA reactivity (Log10)	1.159**	1.050	1.419	.059	.916		

B: Unstandardized regression coefficient; CI: confidence interval; LL: lower limit; UL: upper limit; *SE B*: standard error of the coefficient; β : standardized coefficient; *R*²: coefficient of determination; $\blacktriangle R^2$: adjusted *R*².

***P* < 0.05

BCG, Bacillus Calmette–Guérin vaccine

550

551 Figure 1: Flow Chart of patient enrollment, follow up and analysis

552

553 Figure 2: Antibody levels from Phase 1 in specimens obtained early during the pandemic

554 (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for

555 symptomatic and asymptomatic participants.

556

557 Figure 3: Simple correlation analysis of HCW with positive reactivity for anti- spike antibody

558 in Phase 1 with baseline characteristics and symptoms

559

560 Figure 4: Simple correlation analysis of rate of decay of anti-spike antibodies between both

561 phases with baseline characteristics and symptoms

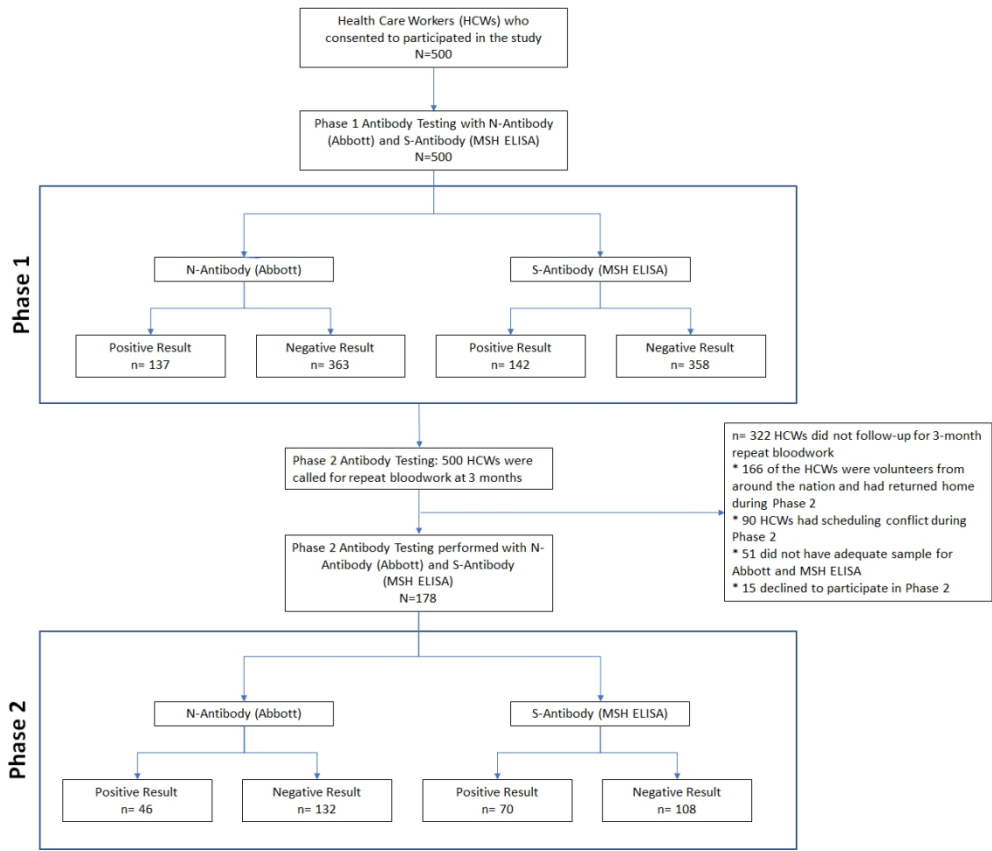
562

563 Figure 5: Paired comparison between rate of decay of anti-spike antibody titres and patient

564 characteristics

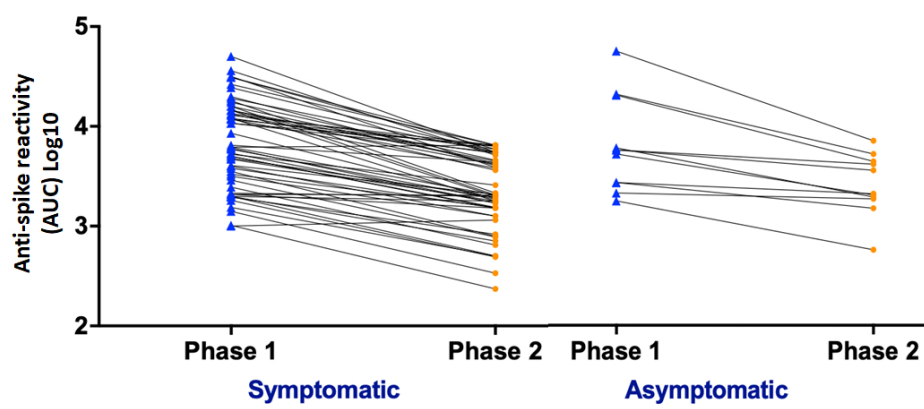
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Flow Chart of patient enrollment, follow up and analysis

340x286mm (96 x 96 DPI)



Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

295x129mm (96 x 96 DPI)

ELISA reactivity in Phase 1 (Log10)	R	1										
	Sig.											
Age	R	0.269**	1									
	Sig.	0.001	-									
Gender	R	0.106	0.050	1								
	Sig.	0.209	0.552	-								
Comorbidities	R	0.130	0.468**	0.045	1							
	Sig.	0.123	0.000	0.596	-							
BCG vaccine	R	0.046	0.014	-0.129	0.084	1						
	Sig.	0.585	0.869	0.125	0.317	-						
Fever	R	0.319**	0.024	-0.089	0.018	0.060	1					
	Sig.	0.000	0.777	0.292	0.828	0.479	-					
URS	R	0.278**	0.039	0.059	0.069	-0.214*	0.464**	1				
	Sig.	0.001	0.645	0.486	0.413	0.010	0.000	-				
GI symptoms	R	0.204*	0.030	0.068	0.029	0.024	0.344**	0.291**	1			
	Sig.	0.015	0.723	0.423	0.733	0.775	0.000	0.000	-			
Number of symptoms	R	0.310**	-0.015	0.070	0.026	0.019	0.708**	0.665**	0.630**	1		
	Sig.	0.000	0.856	0.405	0.762	0.825	0.000	0.000	0.000	-		
Days of symptoms	R	0.434**	0.036	0.019	0.121	-0.038	0.422**	0.605**	0.347**	0.681**	1	
	Sig.	0.000	0.671	0.817	0.151	0.656	0.000	0.000	0.000	0.000	-	
Degree of HCW exposure	R	-0.145	-0.215**	-0.217**	-0.177*	0.017	0.017	0.034	0.000	0.039	-0.085	1
	Sig.	0.083	0.010	0.009	0.034	0.838	0.844	0.690	0.998	0.640	0.314	-

ELISA reactivity in Phase 1 (Log10)

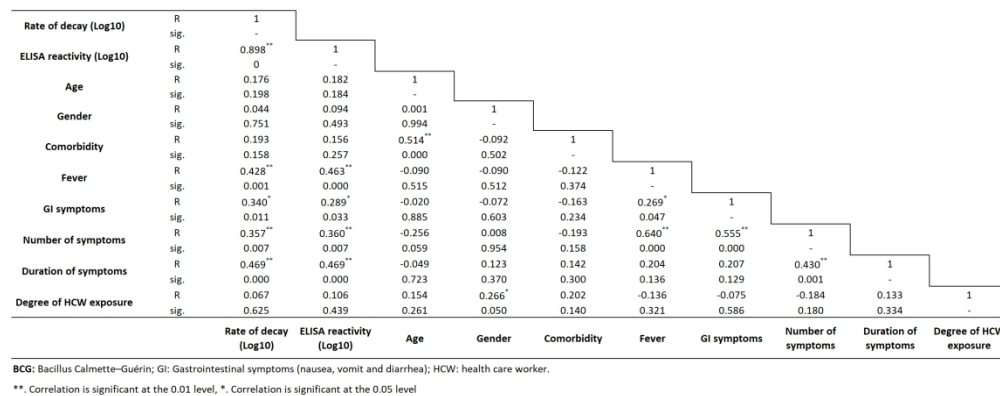
Age Gender Comorbidities BCG vaccine Fever URS GI symptoms Number of symptoms Days of symptoms Degree of HCW exposure

BCG, Bacillus Calmette-Guérin; URS, upper respiratory symptoms; GI, Gastrointestinal symptoms (nausea, vomit and diarrhoea); HCW, health care worker.

**, Correlation is significant at the 0.01 level, *, Correlation is significant at the 0.05 level.

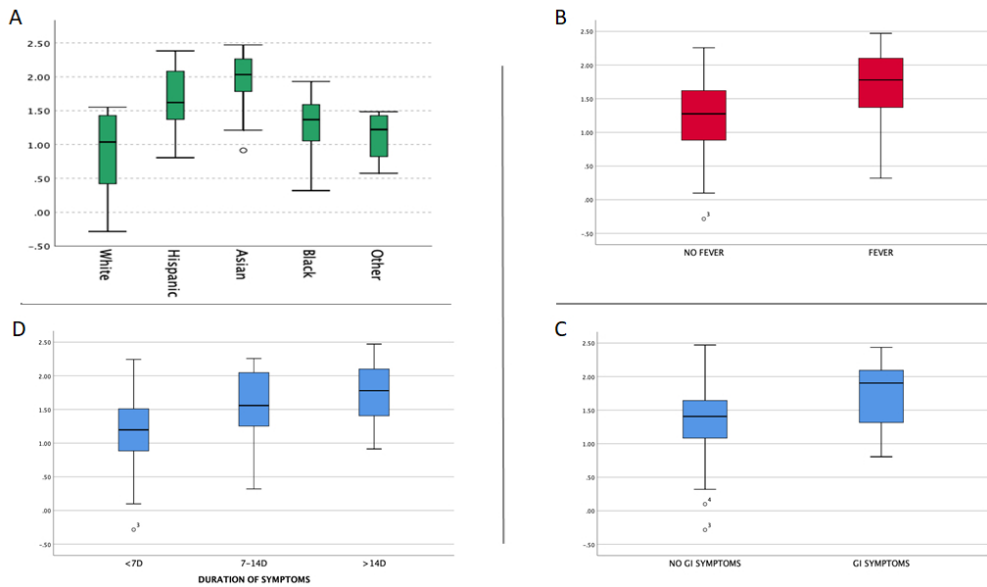
Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms.

767x285mm (96 x 96 DPI)



Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms.

686x269mm (96 x 96 DPI)



Paired comparison between rate of decay of anti-spike antibody titres and patient characteristics.

270x161mm (96 x 96 DPI)

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohort reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	7

Methods

1	Study design	#4	Present key elements of study design early in the paper	7
2				
3	Setting	#5	Describe the setting, locations, and relevant dates, including	7
4			periods of recruitment, exposure, follow-up, and data collection	
5				
6				
7	Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of	7
8			selection of participants. Describe methods of follow-up.	
9				
10				
11	Eligibility criteria	#6b	For matched studies, give matching criteria and number of	7
12			exposed and unexposed	
13				
14				
15	Variables	#7	Clearly define all outcomes, exposures, predictors, potential	7
16			confounders, and effect modifiers. Give diagnostic criteria, if	
17			applicable	
18				
19				
20	Data sources /	#8	For each variable of interest give sources of data and details of	8
21	measurement		methods of assessment (measurement). Describe	
22			comparability of assessment methods if there is more than one	
23			group. Give information separately for for exposed and	
24			unexposed groups if applicable.	
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28	Bias	#9	Describe any efforts to address potential sources of bias	16
29				
30				
31	Study size	#10	Explain how the study size was arrived at	9
32				
33	Quantitative	#11	Explain how quantitative variables were handled in the	7-9
34	variables		analyses. If applicable, describe which groupings were chosen,	
35			and why	
36				
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38	Statistical	#12a	Describe all statistical methods, including those used to control	9
39	methods		for confounding	
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44	Statistical	#12b	Describe any methods used to examine subgroups and	9
45	methods		interactions	
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48	Statistical	#12c	Explain how missing data were addressed	9
49	methods			
50				
51				
52	Statistical	#12d	If applicable, explain how loss to follow-up was addressed	9
53	methods			
54				
55				
56	Statistical	#12e	Describe any sensitivity analyses	9
57	methods			
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Results

Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	30
Participants	#13c	Consider use of a flow diagram	
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9,23-24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	9,23-26
9,23-26			
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	4, 10
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	11
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
Main results	#16b	Report category boundaries when continuous variables were categorized	10
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11

Other analyses	#17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-13
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13-14
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	16
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	15-16
Generalisability	#21	Discuss the generalisability (external validity) of the study results	16-17
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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BMJ Open

Longitudinal Humoral Antibody Response to SARS-CoV-2 Infection among Health Care Workers in a New York City Hospital

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Primary Subject Heading:	Health services research
Secondary Subject Heading:	Infectious diseases, Immunology (including allergy)
Keywords:	COVID-19, INFECTIOUS DISEASES, IMMUNOLOGY



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1 Longitudinal Humoral Antibody Response to SARS-CoV-2 Infection
2 among Health Care Workers in a New York City Hospital

3
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13 Keywords: SARS-CoV-2 Infection, anti-spike antibody, decay rate, COVID-19

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17 Word count: 3396

18
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26

Abstract

Objective

Dynamics of humoral immune responses to SARS-CoV-2 antigens following infection suggests an initial decay of antibody followed by subsequent stabilization. We aim to understand the longitudinal humoral responses to SARS-CoV-2 nucleocapsid (N) protein and spike (S) protein and to evaluate their correlation to clinical symptoms among healthcare workers (HCW).

Design

A prospective longitudinal study.

Setting

This study was conducted in New York City Public Hospital in the South Bronx, New York.

Participants

HCWs participated in Phase 1 (N=500) and were followed up 4 months later in Phase 2 (N=178) of the study. They underwent SARS-CoV-2 PCR and serology testing for N and S protein antibodies, in addition to completion of an online survey in both phases. Analysis was performed on the 178 participants that participated in both phases of the study.

Primary outcome measure

Evaluate longitudinal humoral responses to viral N (qualitative serology testing) and Spike protein (quantitative MSH-ELISA to detect Receptor binding domain and full-length S reactive antibodies) by measuring rate of decay.

Results

Anti-N antibody positivity was 27% and anti-S positivity was 28% in Phase 1. In Phase 1 anti-S titers were higher in symptomatic (6754 [5177-8812]) than in asymptomatic positive subjects (5803 [2825-11920]). Marginally higher titers (2382 [1494-3797]) were seen in asymptomatic compared to the symptomatic positive subgroup (2198 [1753-2755]) in Phase

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2. A positive correlation was noted between age ($R=0.269$, $p<0.01$), number ($R=0.310$, $p<0.01$) and duration of symptoms ($R=0.434$, $p<0.01$), and Phase 1 anti-S antibody titer. A strong correlation ($R=0.898$, $p<0.001$). was observed between Phase 1 titers and decay of anti-S antibody titers between the two phases. Significant correlation with rate of decay was also noted with fever ($R=0.428$, $p<0.001$), GI symptoms ($R=0.340$, $p<0.05$), and total number ($R=0.357$, $p<0.01$) and duration of COVID-19 symptoms ($R=0.469$, $p<0.001$).

Conclusions

Higher initial anti-S antibody titers were associated with larger number and longer duration of symptoms as well as a faster decay between the two time points.

Strengths and limitations of this study

- The strength of our study is the longitudinal design with serial sampling to determine humoral response to SARS-CoV-2 infection from consenting Health Care Workers during the pandemic.
- This study collected serial detailed characteristics of symptomatic and asymptomatic Health Care Workers to correlate with durability and decay of humoral response.
- This study is limited by representation of only a single institution's data and the possibility of recall bias to the responses on the online survey.
- Our cohort for Phase 2 was smaller than Phase 1, due to discontinuation of volunteer healthcare workers from the surge period.

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Introduction

In light of the unprecedented Coronavirus Disease 2019 (COVID-19) pandemic, understanding the role of the immune system in countering the viral infection is critical not just to design effective antiviral strategies but also to aid us in taking appropriate public health decisions. The early publication of the viral genome led to a rapid development of many nucleic acid based diagnostic assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections. While nucleic acid-based tests are widely employed in the diagnosis of acute (current) SARS-CoV-2 infections, they are often limited in their clinical utility in identifying past infections or assess the level of immunity to SARS-CoV-2 within the communities. Evaluation of antibody responses is the other well-known modality used in a clinical setting that can detect both current, and past infections and is the preferred approach for surveillance to determine the true prevalence of infections. The currently available serological assays for SARS-CoV-2 target either the viral nucleoprotein (N) or the spike surface protein (S) antigens. The S-protein, which contains the receptor binding domain (RBD), binds to host cells via the angiotensin converting-enzyme-2 (ACE2) receptor, followed by membrane fusion^{1,2}. The spike is the target of most neutralizing antibodies³⁻⁵, while the N plays an important role in transcription enhancement and viral assembly⁶. Studies have demonstrated that antibodies against the N and S appeared around the same time - between day 8 and day 14 after the onset of symptoms with antibodies to the N being more sensitive than anti S antibodies for detecting early infection⁷. Neutralizing antibodies confer protective immunity and can be detected in most infected individuals 10-15 days following the onset of COVID-19 symptoms and remain elevated following initial viral clearance⁸⁻¹². There is compelling evidence to suggest that serological assays for anti-S antibodies predict neutralizing activity, in contrast to N based assays^{11,13}.

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96 The detailed characterization of the dynamics of humoral immune responses to the SARS-
97 CoV-2 viral antigens following infection is still ongoing and early evidences suggest an initial
98 decay of antibody followed by stabilization at a certain level^{11,14-18}. These dynamics are likely
99 driven by an initial expansion of plasmablasts which produce large amounts of antibody but
100 die off quickly followed by a slower decay of antibody titers (the half-life of IgG is
101 approximately three weeks) which then transitions into a steady state level of antibody
102 produced by long-lived plasma cells¹⁹. However, it is currently unknown, if the magnitude of
103 the initial expansion of plasmablast and the associated antibody titers are correlated with the
104 steady state level of serum antibody produced by long-lived plasma cells. This is an important
105 question since steady state antibody levels may provide superior protection from re-
106 infection^{20,21}.

107

108 Specifically, there is currently a paucity of information on the kinetics of antibody decay among
109 health care workers (HCW). It is suspected that SARS-CoV-2 infections among HCW are
110 usually asymptomatic or mildly symptomatic and frequently associated with either
111 underreporting of symptoms or heterogenous PCR and/or serologic diagnostics leading to most
112 of them going undetected or unrecognized²². A large cohort study of HCWs in the greater New
113 York City (NYC) area showed a seroprevalence of SARS-CoV-2 antibodies of 13.7%²³. Our
114 own data of anti N antibody screening among HCW at a New York City public hospital in the
115 Bronx following the first “surge” of COVID-19 in May 2020, found that SARS-CoV-2
116 seroprevalence was at 27%²⁴. Understanding the longitudinal kinetics of SARS-CoV-2
117 antibody response and the effectiveness of commercial antibody measurement assays is crucial
118 to correctly determine infection rates, sero-prevalence and true sero-reversion rates in both

infected and vaccinated individuals – and to better understand protection associated with sero-positivity.

In this study, we aimed to investigate the longitudinal humoral responses to viral N and the spike protein and to evaluate their correlation to clinical symptoms and baseline characteristics of the HCW cohort. We also evaluated if initial high antibody levels correlated with high antibody titers at steady state.

Methods

Study setting and population

This is a prospective longitudinal study done in two phases after receiving Institutional Review Board approval (IRB # 20-009). The Phase 1 study was conducted in May/June, 2020 and the Phase 2 was completed August/September 2020. The cohort included HCWs who worked at the New York City Public Hospital in the South Bronx. Information about the study was disseminated among health care workers via hospital’s intranet bulletins, by research staff approaching on duty staff and handing out study flyers and introducing the study in multiple department meetings. The HCWs who had participated in Phase 1 were called individually to schedule an appointment with research staff for blood-work and survey completion for Phase 2 study.

In the Phase 1 of the study, after informed consent, participants underwent qualitative serology testing (Abbott Architect SARS-CoV-2 IgG Assay, Abbott Park, IL 60064 USA)²⁵ and a nasopharyngeal swab for SARS-CoV-2 (Bio-Reference Laboratories, Inc., Elmwood Park, NJ, USA). They also completed an initial online survey on demographics, symptoms of COVID-19 including duration, and healthcare/community exposure. An extra sample was collected and

stored at -80°C for subsequent analysis. These samples were processed using a quantitative enzyme-linked immunosorbent assay (ELISA), developed by Mount Sinai Health System (MSH ELISA)^{26,27}, that correlates well with virus neutralization, to detect RBD and full-length spike (S) reactive antibodies. In Phase 2 of the study, consenting HCWs underwent qualitative and quantitative serology assessment by Abbott and MSH ELISA tests, respectively. They also completed a follow-up online survey including information about demographics, interval SARS-CoV2 PCR positivity and healthcare/community exposure.

Antibody assays

The Abbott Architect assay uses a qualitative chemiluminescent microparticle immunoassay technology targeting the N antigen of the virus with a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6% (CI 99–99.9%)²⁵. The MSH ELISA consists of an initial ELISA using serum or plasma to detect specific IgG against the RBD of SARS-CoV-2 at a single dilution, followed by quantitative titrations of presumptive positives in a confirmatory ELISA against full length SARS-CoV-2 spike protein (S)²⁸. The positive result from the spike ELISA is reported as antibody at a titer of 1:80 or higher. Test performance assessment revealed that PCR+ samples were 94 % positive and all negative samples returned a negative result for 100% negative agreement²⁹.

Survey

The open-access online SurveyMonkey tool (SurveyMonkey, San Mateo, CA, U.S.A.; <http://www.surveymonkey.com>) was used to create and administer our survey to participating HCWs. The survey in both phases was developed with feedback from the Research Team. Open text questions were minimized. Preliminary versions of the survey were piloted among a focus group of 10 healthcare providers and their feedback about length, flow, ease of response,

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2
3 169 and acceptability to respondents was incorporated to finalize the version administered to the
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5 170 participants. The online survey was accessed by a unique identification number assigned to
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8 171 each participant, blinded to the research team to ensure confidentiality. The Phase 1 survey was
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10 172 designed to capture demographics and current medical history, number and duration of
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12 173 symptoms of COVID-19 infection (exposure during the pandemic prior to Phase 1),
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14 174 domestic/international travel, and healthcare and community exposure²⁴. The risk of exposure
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17 175 in the healthcare setting and community exposure was determined based on CDC guidelines³⁰.
18
19 176 The Phase 2 survey requested information on new comorbidities, persistent COVID-19
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21 177 symptoms (cough, shortness of breath, anosmia, ageusia, myalgia, nausea, and/or diarrhea),
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23 178 interim testing via antibody and/or reverse transcription polymerase chain reaction (RT -PCR)
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26 179 (if present) and their result (positive/negative), presence of positive SARS-CoV-2 PCR results
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29 180 in the preceding months (exposure after Phase 1 sample collection), interim
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31 181 domestic/international travel, and continued use of personal protective equipment (PPE). Both
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33 182 surveys have been attached as online supplemental materials (Supplementary materials
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35 183 section).

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40 185 **Statistical analysis**
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42 186 Convenience sampling design was adapted to recruit participants with a goal of 500
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44 187 participants. Descriptive statistics were used to summarize the baseline characteristics of the
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46 188 cohort and key study outcome variables. Categorical variables were compared by the Chi-
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49 189 squared test, while continuous variables were compared by a Student's t-test. The spike
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51 190 antibody titers were described as geometric means. Correlations were calculated using standard
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53 191 Pearson and Spearman correlation. Multiple linear regression was applied to determine the
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56 192 predictors of log10 rate of decay from Phase 1 to Phase 2 of anti-spike antibodies. A p-value

of <0.05 was considered significant. All statistical analyses were performed using SPSS version 27 (IBM, USA).

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Results

For Phase 1 of the study, 500 healthcare workers underwent both PCR and serology testing. Of these, 137 were positive by for anti-N antibody (Abbott) and 142 were positive by the MSH ELISA. In the Phase 2, 178 participants from the initial cohort. The interval between Phase 1 and Phase 2 was 133 ± 21 days. The details of patient enrolment are described in **Figure 1**. While 46 of the 178 tested subjects remained positive for the anti-N antibody (Abbott), 70 were positive by the MSH ELISA in the second phase. Anti -spike titers of the 5 subjects in the first phase were close to the cut off for positivity. Twenty-two subjects who were negative for anti-N antibody in Phase 2 had positive titers of anti-RBD and anti-spike antibodies, though lower than their Phase 1 levels. Among the subjects who participated in the Phase 1 and Phase 2 study, 68 were positive in both phases by the MSH ELISA, 110 were negative in both phases and 2 were positive only in Phase 2 with previously negative results in Phase 1.

The baseline characteristics of study participants who were positive by MSH ELISA in both phases ($n=68$) and those who were negative in both phases ($n=108$) are shown in **Table 1**. The mean age of the participants was 44.7 ± 12.4 years, and 63.1% were female. Overall, 30.7% of the HCWs were Latinx, 29.5% were Asian, 16.5% were Black and 17.6% were White. COVID-19 related symptoms were present in 83.8% (57) of the subjects who were positive in both phases, while only 42.6% (46) of the subgroup who had negative antibodies in both phases

admitted to symptoms prior to Phase 1. The duration of symptoms prior to Phase 1 was longer among the symptomatic positive group (48.3% for >14 days) in comparison to symptomatic negative group (17.8% for >14 days). The mean duration of symptoms to Phase 1 testing in the symptomatic positive sub cohort was 47.9 ± 16.0 days. Persisting symptoms of COVID-19 were reported in 19 (27.9%) subjects from the cohort with positive antibodies in both phases.

Clinical characteristics and seropositivity to spike protein in both phases

Table 2 describes the characteristics of the symptomatic and asymptomatic subjects who were positive for anti-spike antibody in both phases. Baseline characteristics were comparable between the groups and no difference either in the healthcare or community exposure or in the location of work (ED/Inpatient/intensive care unit, OR etc.) between the two groups was observed. Titers of anti-spike antibodies (geometric mean area under the curve (AUC)) were higher in symptomatic subjects than in asymptomatic positive subjects (6754 AUC vs. 5803 AUC) in Phase 1. However, in the Phase 2 analysis we observed marginally higher titers in the asymptomatic subgroup compared to the symptomatic subgroup (2383 AUC vs. 2198 AUC). Figure 2 illustrates the symptomatic and asymptomatic antibody levels of anti-spike antibodies. The rate of decay was higher in the symptomatic subgroup (geometric mean 32.96 per day) compared to the asymptomatic (geometric mean 23.42 per day) suggesting delayed antibody/kinetics in the asymptomatic cohort.

Phase 1 anti-spike antibody titer and clinical correlations

A Pearson’s product and Spearman’s rank-order correlation was run to assess the relationship between cohort characteristics including age, gender, comorbidities, number of symptoms of COVID-19, healthcare exposure and Phase 1 anti-spike titers in our cohort (**Figure 3**). One hundred-forty-three subjects with a positive test in Phase 1 were included in the analysis.

Scatter plot analysis showed a monotonic relationship between the variables. A statistically significant weak positive correlation was observed between age and Phase 1 anti-spike antibody titers ($R=0.269$, $p<0.01$). Moderate positive correlation was present between presence of fever ($R=0.319$, $p<0.01$), number of symptoms ($R=0.310$, $p<0.01$) and days of symptoms ($R=0.434$, $p<0.01$) and anti-spike antibody titer; and weak positive correlation was observed with upper respiratory symptoms ($R=0.278$, $p<0.01$) and gastrointestinal (GI) symptoms ($R=0.204$, $p<0.05$) with anti-spike antibody titers.

Correlation of rate of decay of anti-spike antibody titers from Phase 1 to Phase 2 and clinical characteristics

Results of Pearson correlation to assess the relationship between cohort characteristics including Phase 1 anti-spike antibody titers, age, gender, comorbidities, symptoms of COVID-19, number of symptoms of COVID-19, healthcare exposure and decay of anti-spike titers between the two phases in our cohort is shown in **Figure 4**. A strong positive statistically significant correlation was observed between Phase 1 titers and decay of anti-spike antibody titers between the two phases ($R=0.898$, $p<0.001$). Medium positive correlation was observed between presence of fever ($R=0.428$, $p<0.001$), GI symptoms ($R=0.340$, $p<0.05$), number of symptoms ($R=0.357$, $p<0.01$), duration of symptoms ($R=0.469$, $p<0.001$) with decay of anti-spike antibody titers between the two phases respectively.

A pairwise comparison was performed between rate of decay of anti-spike antibody titers and patient characteristics (**Figure 5**). Rate of decay by gender was comparable (male; 30.73 AUC/day vs. female; 34.68 AUC/day, $p=0.413$). Asian (86.0 AUC/day) demonstrated a higher rate of decay compared with Whites (7.2 AUC/day) and Blacks (19.61 AUC/day) individuals;

while Latinx (47.28 AUC/day) race had higher rate of decay compared with White (7.2 AUC/day) individuals. Subjects with fever had a higher rate of decay than those who did not report fever (53.08 AUC/day vs.16.14 AUC/day, $p<0.01$). Similarly subjects with GI symptoms had a higher rate of decay than those without (55.81 AUC/day vs.21.94 AUC/day, $p<0.05$). Subjects with symptoms restricted to less than seven days demonstrated a lower rate of decay when compared with symptomatic subjects over 7-14 days (13.60 AUC/day vs. 36.12 AUC/day, $p<0.05$) and when compared with symptomatic subjects with more than 14 days (13.60 AUC/day vs. 59.72 AUC/day, $p<0.001$). This finding was statistically significant. No difference was found when degree of exposure (High/Moderate: 28.18 AUC/day vs. Mild: 34.78 AUC/day, $p=0.395$) or job role (physician: 29.57 AUC/day vs. nurse: 53.59 AUC/day vs. Other: 26.83 AUC/day; $p=0.361$) were compared to rate of decay.

Predictors of rate of decay from Phase 1 to Phase 2 of anti-spike antibodies

Multiple linear regression analysis to predict the rate of decay with respect to age, Bacillus Calmette Guerin vaccination, number of symptoms, and Phase 1 (log10) anti-spike antibody titers is shown in **Table 3**. On the basis of a linear regression model that included the participants age, history of BCG vaccination, total number of COVID-19 symptoms and the Phase 1 concentration of log 10 spike antibody titers, the estimated change (decay) was 23.6 AUC/day when age was centred at median (42.6 years), there was positive history of BCG vaccinations, the total number of COVID-19 symptoms were centred at a median of 4, and the geometric mean of the log₁₀ spike antibody titer was 3.78.

Discussion

With the COVID-19 pandemic showing no signs of abating, healthcare workers at the epicentre are at risk of infection due to occupational exposure as well as community

293 exposure. Sero-surveillance is the foundation for determining the scale and rate of exposures.
294 With a multitude of serological assays getting emergency use approval from FDA,
295 interpretation of the results of these assays and their clinical significance remains
296 challenging. It is critical to understand the timing of the antibody response for acute
297 interpretation. Confidence in analytical specificity of the assay is a critical requirement in
298 measurement of the specific antibody responses. Recent studies have confirmed that anti
299 spike titers especially anti-RBD titers can serve as surrogates for virus neutralization^{31,32}. The
300 Abbott SARS-CoV-2 IgG assay that targets antibodies to the nucleoprotein has a reported
301 specificity and sensitivity of greater than 99% at 14 days or more following symptom onset
302 and these measurements are not indicative or correlated to virus neutralization titers³³. In
303 comparison, the MSH ELISA targets the full-length S protein including RBD, a major target
304 for neutralizing antibodies and has demonstrated excellent correlation to virus
305 neutralization^{11,26}. Longitudinal measurements of antibody levels have revealed that anti-N
306 and anti S IgG antibodies continue to increase until the third week post symptoms and an
307 approach that combines the detection of both of these antibodies would precisely detect
308 almost 100% of all infectious exposures³⁴. In our study, the mean number of days after
309 symptoms to testing in Phase 1 was 47 days suggesting a higher likelihood of accuracy of the
310 utilized assay.

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312 Longitudinal blood sampling among HCWs working at a public hospital which was at the
313 epicentre of the pandemic in NYC allowed for analysis of kinetics of anti-S and anti-N
314 antibody responses. At two months after the first surge of infections, anti-N antibodies were
315 detected in 27% and anti-S antibodies in 28% of participating HCWs. After an interval of
316 four months, it is not surprising to note that among the participants who returned, 26%
317 remained positive for anti N antibodies, while 31% of the previously anti-N antibody positive

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3 318 subjects tested negative in phase 2. On the other hand, a similar analysis of the anti-S
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5 319 antibodies levels, confirmed that all the previously positive retested subjects continued to
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7 320 remain positive, albeit with lower titers. That being said, we acknowledge that the decline of
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9 321 N antibodies in our cohort could be due to the Abbott assay being less sensitive to describe
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11 322 the dynamics of N antibodies over time compared to other assays, like Roche, Siemens and
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13 323 Diasorin. Muecksch et al. demonstrated in their longitudinal analysis of clinical serology
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15 324 assay performance among COVID-19 convalescents, that there is a difference in diagnostic
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17 325 performance among various serologic assays³².
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23 327 COVID-19 related symptoms were significantly associated with positive anti-spike antibodies
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25 328 in both phases, with a similar association with longer duration (>14 days) of symptoms.
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27 329 Previous studies have demonstrated a lower level of IgG response among patients without
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29 330 symptoms or with mild symptoms compared to those with severe and critical disease^{35,36}. A
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31 331 comparison of symptomatic versus asymptomatic subjects who tested positive for anti-spike
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33 332 antibodies in both phases, confirmed that the rate of decay of anti-spike antibody titers were
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35 333 faster in the symptomatic cohort than the asymptomatic subjects, which was seen also in the
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37 334 anti-N antibody kinetics. We observed a faster decay in this group with a lower titer of anti-
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39 335 spike antibodies in Phase 2 compared to the asymptomatic cohort (though the difference was
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41 336 not statistically significant). This could additionally be supported by the finding of fever and
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43 337 GI symptoms contributing to faster decay. Similar results of decreasing neutralizing antibody
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45 338 titer in symptomatic than asymptomatic patients were observed by Choe et al.³⁷
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53 340 Positive correlations for age, presence of fever, upper respiratory symptoms, GI symptoms,
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55 341 total number and duration of symptoms was observed with increased levels of anti-spike titers
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57 342 at Phase 1. Similar results of neutralizing antibody titers were also observed by
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Boonyaratanakornkit et al. wherein they showed higher levels of neutralizing antibody titers were significantly associated with male gender, older adults, higher disease severity and shorter interval from recovery³⁸. Based on a linear regression model with age centred at median (42.6 years), positive history of BCG vaccination, the total number of COVID-19 symptoms centred at a median of 4, and the geometric mean of the log10 anti-spike antibody titer at 3.78, we observed that the rate of decay of these antibody titers was 23.6 AUC/day. Evaluation of other characteristics with rate of decay between Phase 1 and Phase 2 showed a faster reduction in titers in Asian participants and in those with fever and GI symptoms. A slower decrease was noted among patients with shorter duration (<7 days) of symptoms, with no other significant correlation noted with any other baseline demographics or clinical characteristics.

As described above, higher antibody titers are associated with a larger number of symptoms, longer duration of symptoms and – as described by others as well – disease severity in general. We also found that higher initial antibody titers were associated with faster antibody decay during the two time points. Initial antibody responses are driven by short lived plasmablasts, which decay after a few days after producing massive amounts of antibody. IgG has a relatively long half-life of approximately three weeks, but decay is inevitable since the plasmablasts initially producing it disappear. Usually, titers then drop until they reach relatively stable levels of antibody which are maintained by long-lived plasma cells in the bone marrow¹⁹. The two time points described in this study represent the initial peak response and likely the stable level after the initial decay. We found that individuals with higher initial titers had a faster decay rate during the observation period meaning the difference between peak and stable, long-lived antibody levels were larger. This indicates that there is likely no direct correlation between the magnitude of the initial expansion of plasmablasts and the number of long-lived plasma cells that migrate to the bone marrow. It is critical to recognize that steady state antibody titers are

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368 similar between the symptomatic and asymptomatic subgroups, suggesting that mid-term
369 humoral protection might be similar after infection regardless of disease severity.

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371 Our study has the following limitations: First being a single center study with a small
372 convenience sampling method that included a smaller number of participants in Phase 2 of the
373 study. Following the pandemic, the HCWs who had volunteered from around the country were
374 transferred back and thus lost to follow-up. While this did decrease the overall sample size, it
375 is notable that the rates of positive and negative results remained proportional. Secondly, there
376 is a possibility of recall bias in the participant’s responses on the online survey. Lastly, the
377 study findings can underestimate rates of prior infections based on timing of the testing given
378 that antibodies are only transiently detectable following infection.

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380 In conclusion, findings from this study are similar to other studies that have reported that higher
381 magnitude of anti-spike titers may correlate with protection against reinfection, in spite of the
382 observed decay in the antibody levels^{20,21}. Nevertheless, further studies to evaluate the
383 longevity of immunity, especially in context of widespread administration of spike-based
384 vaccine among HCWs would be important in predicting herd immunity to COVID-19
385 infections.

386
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403
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405 analysed the data. E.V. and M.G. assisted with participant follow-up and coordination with
406 the assistance of A.P., U.V., V.M., and M.A.S. The Mount Sinai Health System team, J.M.C.
407 and F.K., performed the measurements for anti-Spike and Anti-RBD antibodies. V.M., U.V.
408 and A.P. were responsible for the clinical care of the research participants and supervised the
409 day-to-day operation and coordination of the study by M.K., V.D., M.A.S., B.Y., V.P.G.,
410 M.G., E.V., and M.G. V.M. and F.K wrote the manuscript and are the guarantors of this work
411 and have full access to all data in the study and take responsibility for the integrity of the data
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Competing Interests: F.K. is listed as a co-inventor on a patent application filed by The Icahn School of Medicine at Mount Sinai relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020), and is currently consulting for Seqirus and Avimex. F.K.’s Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2.

All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not required

Ethics approval: The study protocol was approved by the Institutional Review Board approval (IRB # 20-009, Lincoln Medical Center, Office of the Institutional Review Board approved as per 45 CFR 46 & 21 CFR50,56 under a full board committee and gave its approval on 4/28/2020). All participants provided written informed consent for the use of their data.

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All data relevant to the study has been included in the article.

References

1. Ou X, Liu Y, Lei X, et al. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. *Nat Commun.* 2020;11(1):1620.
2. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veersler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell.* 2020;181(2):281-292.
3. Premkumar L, Segovia-Chumbez B, Jadi R, et al. The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci Immunol.* 2020;5(48):eabc8413.
4. Ni L, Ye F, Cheng ML, et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. *Immunity.* 2020;52(6):971-977
5. Iyer AS, Jones FK, Nodoushani A, et al. Persistence and decay of human antibody responses to the receptor binding domain of SARS-CoV-2 spike protein in COVID-19 patients. *Sci Immunol.* 2020; 5(52):eabe0367.
6. Cong Y, Ulasli M, Schepers H, et al. Nucleocapsid Protein Recruitment to Replication-Transcription Complexes Plays a Crucial Role in Coronaviral Life Cycle. *J Virol.* 2020;94(4):e01925-19.
7. Burbelo PD, Riedo FX, Morishima C, et al. Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients. *medRxiv [Preprint].* 2020;2020.04.20.20071423
8. Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med.* 2020;26(6):845-848.
9. İnandıklioğlu N, Akkoc T. Immune Responses to SARS-CoV, MERS-CoV and SARS-CoV-2. *Adv Exp Med Biol.* 2020;1288:5-12.

1
2
3 466 10. Chaudhuri S, Thiruvengadam R, Chattopadhyay S ,et al. Comparative evaluation of
4
5 467 SARS-CoV-2 IgG assays in India. J Clin Virol. 2020;131:104609.
6
7
8 468 11. Wajnberg A, Amanat F, Firpo A, et al. Robust neutralizing antibodies to SARS-CoV-2
9
10 469 infection persist for months. Science. 2020;370(6521):1227-1230.
11
12 470 12. Crawford KHD, Dingens AS, Eguia R, et al. Dynamics of neutralizing antibody titers
13
14 471 in the months after SARS-CoV-2 infection. J Infect Dis. 2020;jiaa618.
15
16 472 13. Luchsinger LL, Ransegnola BP, Jin DK, et al. Serological Assays Estimate Highly
17
18 473 Variable SARS-CoV-2 Neutralizing Antibody Activity in Recovered COVID-19
19
20 474 Patients. J Clin Microbiol. 2020;58(12)
21
22
23 475 14. Iyer AS, Jones FK, Nodoushani A, et al. Dynamics and significance of the antibody
24
25 476 response to SARS-CoV-2 infection. medRxiv. 2020;2020.07.18.20155374.
26
27
28 477 15. Isho B, Abe KT, Zuo M, et al. Persistence of serum and saliva antibody responses to
29
30 478 SARS-CoV-2 spike antigens in COVID-19 patients. Sci Immunol. 2020;5(52):
31
32 479 eabe5511.
33
34
35 480 16. Wu F, Wang A, Liu m, et al. Neutralizing antibody responses to SARS-CoV-2 in a
36
37 481 COVID-19 recovered patient cohort and their implications. medRxiv,
38
39 482 2020;2020.03.30.20047365.
40
41
42 483 17. Seow J, Graham C, Merrick B, et al. Longitudinal observation and decline of
43
44 484 neutralizing antibody responses in the three months following SARS-CoV-2 infection
45
46 485 in humans. Nat Microbiol. 2020;5(12):1598-1607.
47
48
49 486 18. Grandjean L, Saso A, Torres A, et al. Humoral Response Dynamics Following Infection
50
51 487 with SARS-CoV-2. medRxiv, 2020;2020.07.16.20155663.
52
53
54 488 19. Ellebedy A, Turner J, Kim W, et al. SARS-CoV-2 infection induces long-lived bone
55
56 489 marrow plasma cells in humans. Res Sq [Preprint]. 2020:rs.3.rs-132821.
57
58 490 20. Lumley SF, O'Donnell D, Stoesser NE, et al. Antibody Status and Incidence of SARS-
59
60

- CoV-2 Infection in Health Care Workers. *N Engl J Med.* 2021;384(6):533-540.
21. Hall V, Foulkes S, Charlett A, et al. Do antibody positive healthcare workers have lower SARS-CoV-2 infection rates than antibody negative healthcare workers? Large multi-centre prospective cohort study (the SIREN study), England: June to November 2020. *medRxiv*, 2021;2021.01.13.21249642.
22. Self WH, Tenforde MW, Stubblefield WB, et al. Seroprevalence of SARS-CoV-2 Among Frontline Health Care Personnel in a Multistate Hospital Network. 13 Academic Medical Centers, April-June 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(35):1221-1226.
23. Moscola J, Sembajwe G, Jarrett M, et al. Prevalence of SARS-CoV-2 Antibodies in Health Care Personnel in the New York City Area. *JAMA.* 2020;324(9):893-895.
24. Venugopal U, Jilani N, Rabah S, et al. SARS-CoV-2 seroprevalence among health care workers in a New York City hospital: A cross-sectional analysis during the COVID-19 pandemic. *Int J Infect Dis.* 2021;102:63-69.
25. H Hamilton F, Muir P, Attwood M, et al. Kinetics and performance of the Abbott architect SARS-CoV-2 IgG antibody assay. *J Infect.* 2020;81(6):e7-e9.
26. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med.* 2020;26(7):1033-1036.
27. Stadlbauer D, Amanat F, Chromikova V, et al. SARS-CoV-2 Seroconversion in Humans: A Detailed Protocol for a Serological Assay, Antigen Production, and Test Setup. *Curr Protoc Microbiol.* 2020;57(1):e100.
28. Amanat F, Stadlbauer D, Strohmeier S, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. *Nat Med.* 2020;26(7):1033-1036.
29. Stadlbauer D, Tan J, Jiang K, et al. Repeated cross-sectional sero-monitoring of SARS-CoV-2 in New York City. *Nature.* 2021;590(7844):146-150.

- 516 30. Garcia M, Lipskiy N, Tyson J, Watkins R, Esser ES, Kinley T. Centers for Disease
517 Control and Prevention 2019 novel coronavirus disease (COVID-19) information
518 management: addressing national health-care and public health needs for standardized
519 data definitions and codified vocabulary for data exchange. *J Am Med Inform Assoc.*
520 2020;27(9):1476-1487
- 521 31. Therrien C, Serhir B, Bélanger-Collard M, et al. Multicenter Evaluation of the Clinical
522 Performance and the Neutralizing Antibody Activity Prediction Properties of ten high
523 throughput serological assays used in Clinical Laboratories. *J Clin Microbiol.*
524 2020;JCM.02511-20.
- 525 32. Muecksch F, Wise H, Batchelor B, et al. Longitudinal Serological Analysis and
526 Neutralizing Antibody Levels in Coronavirus Disease 2019 Convalescent Patients. *J*
527 *Infect Dis.* 2021;223(3):389-398.
- 528 33. Rosadas C, Randell P, Khan M, McClure MO, Tedder RS. Testing for responses to the
529 wrong SARS-CoV-2 antigen? *Lancet.* 2020;396(10252):e23.
- 530 34. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses
531 in COVID-19 patients. *Emerg Microbes Infect.* 2020;9(1):940-948.
- 532 35. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of
533 asymptomatic SARS-CoV-2 infections. *Nat Med.* 2020; 26(8):1200-1204.
- 534 36. Wang Y, Zhang L, Sang L, et al. Kinetics of viral load and antibody response in relation
535 to COVID-19 severity. *J Clin Invest.* 2020;130(10):5235-5244.
- 536 37. Choe PG, Kang CK, Suh HJ, et al. Waning Antibody Responses in Asymptomatic and
537 Symptomatic SARS-CoV-2 Infection. *Emerg Infect Dis.* 2021;27(1):327-329.
- 538
- 539 38. Boonyaratanakornkit J, Morishima C, Selke S, et al. Clinical, laboratory, and temporal
540 predictors of neutralizing antibodies to SARS-CoV-2 after COVID-19. *medRxiv.* 2020;

541 2020.10.06.20207472.

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Table 1: Broad characteristics among health care workers assessed for antibody reactivity to spike SARS-CoV-2 protein in Phase 1 and Phase 2

	Overall †	Spike ELISA (AUC) positive in both phases	Negative Reactivity to spike (AUC) in both phases	p value
	N=176	n=68	n=108	
Age, years	44.7+12.4	42.9+11.9	45.8+12.7	0.099
Female, Gender	111 (63.1%)	40 (58.8%)	71 (65.7%)	0.467
Race				0.666
Latinx	54 (30.7%)	21 (30.9%)	33 (30.6%)	
Asian	52 (29.5%)	18 (26.5%)	34 (31.5%)	
Black	29 (16.5%)	15 (22.1%)	14 (13.0%)	
White	31 (17.6%)	10 (14.7%)	21 (19.4%)	
Other	10 (5.7%)	4 (5.9%)	6 (5.9%)	
Comorbidities	54 (30.7%)	25 (36.8%)	29 (26.9%)	0.214
BCG vaccine received in childhood	87 (49.4%)	35 (51.5%)	52 (48.1%)	0.902
COVID-19 related symptoms prior to Phase 1	103 (58.5%)	57 (83.8%)	46 (42.6%)	<.001
Duration of symptoms				<.001
<7 days	48 (46.6%)	18 (31.0%)	30 (66.7%)	
7-14 days	19 (18.4%)	12 (20.7%)	7 (15.6%)	
>14 days	36 (35.0%)	28 (48.3%)	8 (17.8%)	
Time from symptom to positive result, days	45.7+19.9	47.9+16.0	42.9+24.1	0.062
RT-PCR positive result for SARS-CoV-2 prior to Phase 1	51 (29.0%)	49 (72.1%)	2 (1.9%)	<.001
RT-PCR positive result for SARS-CoV-2 during Phase 1	14 (8.0%)	13 (19.1%)	1 (0.9%)	<.001
Persisting symptoms from COVID-19	25 (14.2%)	19 (27.9%)	6 (5.6%)	<.001
Nature of work				0.306
Physicians	81 (46.0%)	29 (42.6%)	52 (51.5%)	
Nurses	29 (16.5%)	15 (22.1%)	14 (13.0%)	
Others	64 (36.4%)	24 (35.3%)	40 (39.6%)	
Hospital areas worked in:				
Emergency department/Inpatient units	118 (67.0%)	50 (73.5%)	68 (63.0%)	0.141
Ambulatory care/Clinics	72 (40.9%)	27 (39.7%)	45 (41.7%)	0.631
Administration/Non-clinical care areas	24 (13.6%)	9 (13.2%)	15 (13.9%)	0.867
Community exposure	47 (26.7%)	19 (27.9%)	28 (25.9%)	0.591

Household exposure	39 (22.2%)	17 (25.0%)	22 (20.4%)	0.343
PPE use at work	173 (98.3%)	67 (98.5%)	106 (98.1%)	0.226
Use of facemask outside of the hospital	158 (89.8%)	58 (85.3%)	100 (92.6%)	0.062

Continuous variables are expressed as mean (SD), categorical variables as n (%).
BCG, Bacillus Calmette–Guérin vaccine; PPE, personal protective equipment; RT-PCR, reverse transcription polymerase chain reaction.

† Demographic data is missing for 2 participants from the overall cohort.

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Table 2: Broad characteristics among health care workers with positive antibody reactivity to SARS-CoV-2 spike in both phases

	Overall	Asymptomatic for SARS-CoV-2 infection	Symptomatic for SARS- CoV-2 infection	<i>p</i> <i>value</i>
	n=68	n=11	n=57	
Age, Mean (±SD)	42.9 (±1.45)	44.5 (±3.8)	42.6 (±1.6)	0.557
Female, n (%)	40 (58.8%)	6 (54.5%)	34 (40.4%)	0.502
Race				0.753
Latinx	21 (30.9%)	3 (27.3%)	18 (31.6%)	
Asian	18 (26.5%)	3 (27.3%)	18 (26.3%)	
Black	15 (22.1%)	3 (27.3%)	12 (21.1%)	
White	10 (14.7%)	2 (18.2%)	8 (14.0%)	
Other	4 (5.8%)	0 (0%)	4 (7.0%)	
Comorbidities				
Hypertension	13 (19.1%)	2 (18.2%)	11 (19.3%)	0.650
Diabetes	6 (8.8%)	0 (0%)	6 (10.5%)	0.332
COPD and asthma	13 (19.1%)	1 (9.1%)	12 (21.1%)	0.326
Number of symptoms, median (IQR)	-	-	4.0 (2.0-5.0)	
Length of symptoms				
<7 days	-	-	19 (33.3%)	
7-14 days	-	-	12 (21.1%)	
>14 days	-	-	26 (45.6%)	
Degree of HCW exposure				0.492
High and Moderate	16 (23.5%)	2 (18.2%)	14 (24.6%)	
Minor	52 (76.5%)	9 (81.8%)	43 (75.4%)	
Community exposure	19 (27.9%)	3 (27.3%)	16 (28.1%)	0.635
Household exposure	17 (25.4%)	3 (27.3%)	14 (24.6%)	0.557
Use of facemask outside of hospital	58 (85.3%)	9 (81.8%)	49 (86.0%)	0.722

Principal means of transportation	0.663			
Public	33 (48.5%)	6 (54.5%)	27 (47.7%)	
Private	35 (51.5%)	5 (45.5%)	30 (52.6%)	
Nature of work	0.502			
Physician	29 (42.6%)	4 (36.4%)	25 (43.9%)	
Nurse	15 (22.1%)	2 (18.2%)	13 (22.8%)	
Other	24 (35.3%)	5 (45.5%)	19 (33.3%)	
Hospital areas work in:	0.288			
Emergency department/inpatient units	32 (47.1%)	6 (54.5%)	26 (45.6%)	
Ambulatory care/clinics	9 (13.2%)	2 (18.2%)	7 (12.3%)	
Inpatient and outpatient setting	18 (26.5%)	3 (27.3%)	15 (26.3%)	
Administration/nonclinical care areas	9 (13.2%)	0 (0%)	9 (15.8%)	
Anti-spike reactivity (AUC)				
Reactivity in phase 1, G-Mean (IQR)	6590 (5165-8410)	5803 (2825-11920)	6754 (5177-8812)	0.647
Days from symptoms to first test, Mean (\pm SD)	-	-	47.7 (\pm 1.9)	
Reactivity in phase 2, G-Mean (IQR)	2226 (1824-2718)	2382 (1494-3797)	2198 (1753-2755)	0.980
Days from symptoms to second test			174.5 (\pm 4.1)	
Rate of decay, G-Mean (IQR)	31.14 (22.11-43.87)	23.42 (8.45-64.93)	32.96 (22.73-47.82)	0.382

Continuous variables are expressed as mean (SD) or interquartile range (IQR), categorical variables as n (%).

AUC, area under the curve; COPD, Chronic obstructive pulmonary disease; HCW, health care worker

Table 3: Multiple linear regression analysis of rate of decay for anti-spike antibodies between Phase 1 and Phase 2

Rate of decay (log10)	<i>B</i>	95.0% CI for <i>B</i>		<i>SE B</i>	β	<i>R</i> ²	ΔR^2
		LL	UL				
Model						0.83	0.82
Constant	-3.203**	-3.647	-2.759	.222			
Age (per 10-year change)	.014	-.005	.007	.002	.030		
BCG vaccination	.131**	.030	.310	.046	.121		
Number of symptoms	.013	-.029	.060	.012	.050		
ELISA reactivity (Log10)	1.159**	1.050	1.419	.059	.916		

B: Unstandardized regression coefficient; CI: confidence interval; LL: lower limit; UL: upper limit; *SE B*: standard error of the coefficient; β : standardized coefficient; *R*²: coefficient of determination; ΔR^2 : adjusted *R*².

***P* < 0.05

BCG, Bacillus Calmette–Guérin vaccine

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Figure 1: Flow Chart of patient enrollment, follow up and analysis

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Figure 2: Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

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Figure 3: Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms

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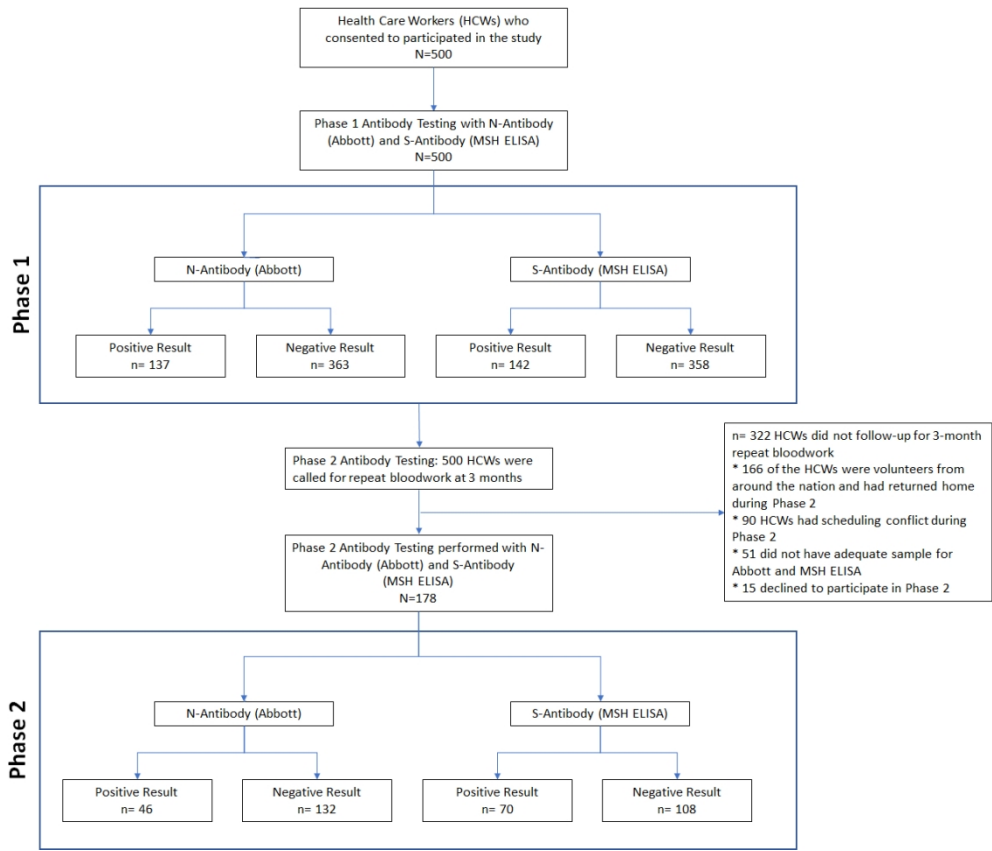
Figure 4: Simple correlation analysis of rate of decay of anti-spike antibodies between both phases with baseline characteristics and symptoms

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Figure 5: Paired comparison between rate of decay of anti-spike antibody titers and patient characteristics

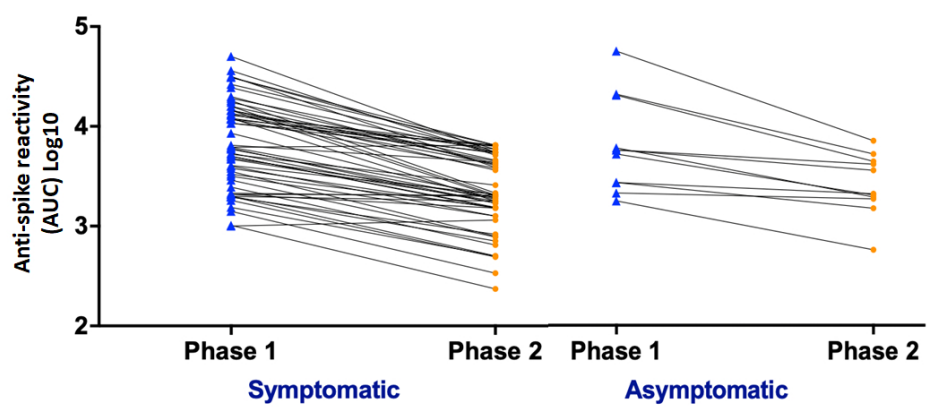
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Flow Chart of patient enrollment, follow up and analysis

340x286mm (96 x 96 DPI)



Antibody levels from Phase 1 in specimens obtained early during the pandemic (May 2020) and Phase 2 in follow up visit (August-October 2020) are shown for symptomatic and asymptomatic participants.

295x129mm (96 x 96 DPI)

ELISA reactivity in Phase 1 (Log10)	R	1										
	Sig.											
Age	R	0.269**	1									
	Sig.	0.001	-									
Gender	R	0.106	0.050	1								
	Sig.	0.209	0.552	-								
Comorbidities	R	0.130	0.468**	0.045	1							
	Sig.	0.123	0.000	0.596	-							
BCG vaccine	R	0.046	0.014	-0.129	0.084	1						
	Sig.	0.585	0.869	0.125	0.317	-						
Fever	R	0.319**	0.024	-0.089	0.018	0.060	1					
	Sig.	0.000	0.777	0.292	0.828	0.479	-					
URS	R	0.278**	0.039	0.059	0.069	-0.214*	0.464**	1				
	Sig.	0.001	0.645	0.486	0.413	0.010	0.000	-				
GI symptoms	R	0.204*	0.030	0.068	0.029	0.024	0.344**	0.291**	1			
	Sig.	0.015	0.723	0.423	0.733	0.775	0.000	0.000	-			
Number of symptoms	R	0.310**	-0.015	0.070	0.026	0.019	0.708**	0.665**	0.630**	1		
	Sig.	0.000	0.856	0.405	0.762	0.825	0.000	0.000	0.000	-		
Days of symptoms	R	0.434**	0.036	0.019	0.121	-0.038	0.422**	0.605**	0.347**	0.681**	1	
	Sig.	0.000	0.671	0.817	0.151	0.656	0.000	0.000	0.000	0.000	-	
Degree of HCW exposure	R	-0.145	-0.215**	-0.217**	-0.177*	0.017	0.017	0.034	0.000	0.039	-0.085	1
	Sig.	0.083	0.010	0.009	0.034	0.838	0.844	0.690	0.998	0.640	0.314	-

ELISA reactivity in Phase 1 (Log10)

Age

Gender

Comorbidities

BCG vaccine

Fever

URS

GI symptoms

Number of symptoms

Days of symptoms

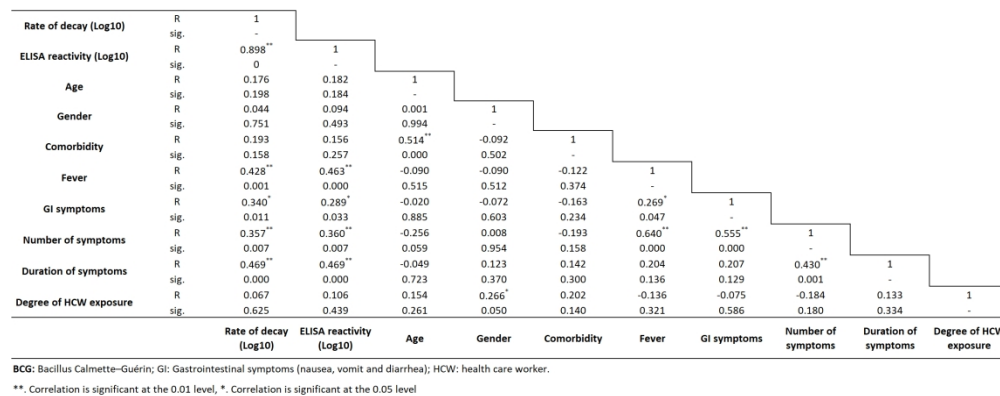
Degree of HCW exposure

BCG, Bacillus Calmette-Guérin; URS, upper respiratory symptoms; GI, Gastrointestinal symptoms (nausea, vomit and diarrhoea); HCW, health care worker.

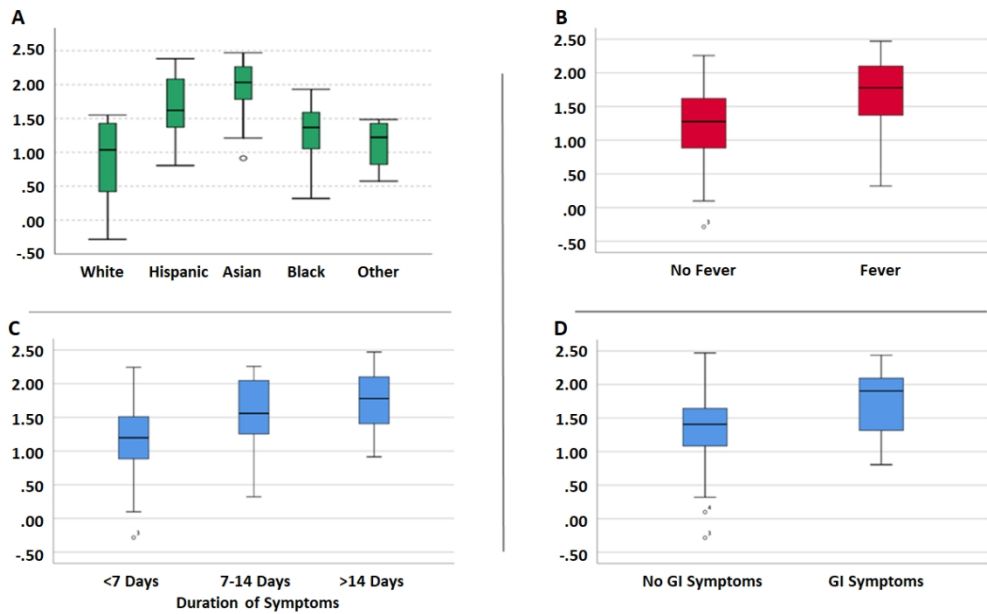
**, Correlation is significant at the 0.01 level, *, Correlation is significant at the 0.05 level.

Simple correlation analysis of HCW with positive reactivity for anti- spike antibody in Phase 1 with baseline characteristics and symptoms.

767x285mm (96 x 96 DPI)



686x269mm (96 x 96 DPI)



Paired comparison between the rate of decay of anti-spike antibody titers and patient characteristics

213x133mm (144 x 144 DPI)

Supplementary Materials Section

Phase 1 Survey

For peer review only

Phase 1 - Survey

	Please enter the unique number that you were given:
	Age, years
	Gender
<input type="checkbox"/>	Male
<input type="checkbox"/>	Female
<input type="checkbox"/>	Non-binary
<input type="checkbox"/>	Prefer Not To Answer
	Do you have any of the following medical conditions?
<input type="checkbox"/>	Hypertension
<input type="checkbox"/>	Diabetes
<input type="checkbox"/>	Heart failure
<input type="checkbox"/>	COPD/Asthma
<input type="checkbox"/>	Chronic kidney disease
<input type="checkbox"/>	Cancer
<input type="checkbox"/>	Rheumatic diseases (i.e. lupus, rheumatoid arthritis, etc)
<input type="checkbox"/>	Not applicable
	Did you experience any of the following symptoms since May 1st 202 to present? (check all that apply):
<input type="checkbox"/>	Fever
<input type="checkbox"/>	Sore throat, cough, sinusitis
<input type="checkbox"/>	Muscle aches, flu like symptoms
<input type="checkbox"/>	Lack of taste
<input type="checkbox"/>	Lack of smell
<input type="checkbox"/>	Nausea/vomiting
<input type="checkbox"/>	Diarrhea
<input type="checkbox"/>	Non of the above
	When did you experience the above symptoms?
<input type="checkbox"/>	May
<input type="checkbox"/>	June
<input type="checkbox"/>	July
<input type="checkbox"/>	August
<input type="checkbox"/>	NA - I did not experience any of the above symptoms
	Approximate duration of symptoms (days)
<input type="checkbox"/>	< 7 days
<input type="checkbox"/>	7-14 days
<input type="checkbox"/>	> 14 days

Tital: Evaluation of Seroprevalence of Antibody to COVID-19 Virus among Healthcare Workers
IRB#20-009

Phase 1 - Survey

<input type="checkbox"/>	NA
<input type="checkbox"/>	Did you get tested from May 1st to present with the following tests:
<input type="checkbox"/>	SARS-CoV2 PCR (nasal swab)
<input type="checkbox"/>	SARS-Cov2 Antibody (blood test)
<input type="checkbox"/>	NA - I was not tested
<input type="checkbox"/>	If you were tested, check the result that was positive:
<input type="checkbox"/>	SARS CoV2 PCR (nasal swab) was positive
<input type="checkbox"/>	SARS CoV2 Antibody (blood test) was positive
<input type="checkbox"/>	NA
<input type="checkbox"/>	Are you experiencing any new or persistent symptoms since March 2020? (check all that apply):
<input type="checkbox"/>	Shortness of breath
<input type="checkbox"/>	Chest pain
<input type="checkbox"/>	Fever
<input type="checkbox"/>	Palpitations
<input type="checkbox"/>	Lack of taste
<input type="checkbox"/>	Lack of smell
<input type="checkbox"/>	Headache
<input type="checkbox"/>	Tingling or pricking sensation of hands/feet
<input type="checkbox"/>	Chronically fatigued
<input type="checkbox"/>	Decreased appetite
<input type="checkbox"/>	No symptoms
<input type="checkbox"/>	Did anyone living with you test positive for SARS-CoV2 PCR from May- August 2020?
<input type="checkbox"/>	Yes
<input type="checkbox"/>	No
<input type="checkbox"/>	Not applicable.
<input type="checkbox"/>	Did you travel in May- August 2020?
<input type="checkbox"/>	Yes
<input type="checkbox"/>	No
<input type="checkbox"/>	Not applicable.
<input type="checkbox"/>	Has there been a change in your living situation since May 2020?
<input type="checkbox"/>	Moved from Apartment to Single Family Home
<input type="checkbox"/>	Moved from Single Family Home to Apartment
<input type="checkbox"/>	No change in living conditions
<input type="checkbox"/>	Has there been a change in your commute to and from work since May 2020?
<input type="checkbox"/>	Yes, I commute using public transportation now
<input type="checkbox"/>	Yes, I commute using private transportation now
<input type="checkbox"/>	No change in the way I commute.

SurveyMonkey

For peer review only - <http://bmjopen.bmj.com/site/about/guidelines.xhtml>
<https://nychealthandhospitals.surveymonkey.com/r/K76R62X>

May 2020

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Phase 1 - Survey	
Have you been diagnosed with any new comorbidity(ies) in the last three months? (check all that apply):	
<input type="checkbox"/>	Diabetes
<input type="checkbox"/>	Asthma
<input type="checkbox"/>	COPD
<input type="checkbox"/>	Coronary Artery Disease
<input type="checkbox"/>	NA
What PPE are you still using while seeing patients?	
<input type="checkbox"/>	N95 only
<input type="checkbox"/>	N95 plus Surgical mask all the time
<input type="checkbox"/>	N95 plus Surgical mask plus Eye protection
<input type="checkbox"/>	N95 + Eye Protection + Face Shield While Seeing COVID-19 Positive Patients
<input type="checkbox"/>	I don't use PPE

Supplementary Materials Section

Phase 2 Survey

For peer review only

Phase 2 - Survey

	Please enter the unique number that you were given:
	Do you have any of the following medical conditions?
<input type="checkbox"/>	Hypertension
<input type="checkbox"/>	Diabetes
<input type="checkbox"/>	Heart failure
<input type="checkbox"/>	COPD/Asthma
<input type="checkbox"/>	Chronic kidney disease
<input type="checkbox"/>	Cancer
<input type="checkbox"/>	Rheumatic diseases (i.e. lupus, rheumatoid arthritis, etc)
<input type="checkbox"/>	Not applicable
	Did you experience any of the following symptoms since May 1st 2020 to present? (check all that apply):
<input type="checkbox"/>	Fever
<input type="checkbox"/>	Sore throat, cough, sinusitis
<input type="checkbox"/>	Muscle aches, flu like symptoms
<input type="checkbox"/>	Lack of taste
<input type="checkbox"/>	Lack of smell
<input type="checkbox"/>	Nausea/vomiting
<input type="checkbox"/>	Diarrhea
<input type="checkbox"/>	Non of the above
	When did you experience the above symptoms?
<input type="checkbox"/>	May
<input type="checkbox"/>	June
<input type="checkbox"/>	July
<input type="checkbox"/>	August
<input type="checkbox"/>	NA - I did not experience any of the above symptoms
	Approximate duration of symptoms (days)
<input type="checkbox"/>	< 7 days
<input type="checkbox"/>	7-14 days
<input type="checkbox"/>	> 14 days
<input type="checkbox"/>	NA
	Did you get tested from May 1st to present with the following tests:
<input type="checkbox"/>	SARS-CoV2 PCR (nasal swab)
<input type="checkbox"/>	SARS-Cov2 Antibody (blood test)
<input type="checkbox"/>	NA - I was not tested
	If you were you tested, check the result that was positive:
<input type="checkbox"/>	SARS CoV2 PCR (nasal swab) was positive
<input type="checkbox"/>	SARS CoV2 Antibody (blood test) was positive
<input type="checkbox"/>	NA

Phase 2 - Survey

Are you experiencing any new or persistent symptoms since March 2020? (check all that apply):

- ☐ Shortness of breath
- ☐ Chest pain
- ☐ Fever
- ☐ Palpitations
- ☐ Lack of taste
- ☐ Lack of smell
- ☐ Headache
- ☐ Tingling or pricking sensation of hands/feet
- ☐ Chronically fatigued
- ☐ Decreased appetite
- ☐ No symptoms

Did anyone living with you tested positive for SARS-CoV2 PCR from May - August 2020?

- ☐ Yes
- ☐ No
- ☐ Not applicable

Did you travel in May - August 2020?

- ☐ Yes
- ☐ No
- ☐ Not applicable

Has there been a change in your living situation since May 2020?

- ☐ Moved from Single Family Home to Apartment
- ☐ Moved from Apartment to Single Family Home
- ☐ No change in living conditions

Has there been a change in your commute to and from work since May 2020?

- ☐ Yes, I commute using public transportation now
- ☐ Yes, I commute using private transportation now
- ☐ No change in the way I commute.

Have you been diagnosed with any new comorbidity(ies) in the last three months? (check all that apply):

- ☐ Diabetes
- ☐ Asthma
- ☐ COPD
- ☐ Coronary Artery Disease
- ☐ NA

Phase 2 - Survey

	What PPE are you still using while seeing patients?
<input type="checkbox"/>	N95 only
<input type="checkbox"/>	N95 plus Surgical mask all the time
<input type="checkbox"/>	N95 plus Surgical mask plus Eye protection
<input type="checkbox"/>	N95 + Eye Protection + Face Shield While Seeing COVID-19 Positive Patients
<input type="checkbox"/>	I don't use PPE

For peer review only

Reporting checklist for cohort study.

Based on the STROBE cohort guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cohort reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

		Reporting Item	Page Number
Title and abstract			
Title	#1a	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	#1b	Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background / rationale	#2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	#3	State specific objectives, including any prespecified hypotheses	7

Methods

1	Study design	#4	Present key elements of study design early in the paper	7
2				
3	Setting	#5	Describe the setting, locations, and relevant dates, including	7
4			periods of recruitment, exposure, follow-up, and data collection	
5				
6				
7	Eligibility criteria	#6a	Give the eligibility criteria, and the sources and methods of	7
8			selection of participants. Describe methods of follow-up.	
9				
10				
11	Eligibility criteria	#6b	For matched studies, give matching criteria and number of	7
12			exposed and unexposed	
13				
14				
15	Variables	#7	Clearly define all outcomes, exposures, predictors, potential	7
16			confounders, and effect modifiers. Give diagnostic criteria, if	
17			applicable	
18				
19				
20	Data sources /	#8	For each variable of interest give sources of data and details of	8
21	measurement		methods of assessment (measurement). Describe	
22			comparability of assessment methods if there is more than one	
23			group. Give information separately for for exposed and	
24			unexposed groups if applicable.	
25				
26				
27				
28	Bias	#9	Describe any efforts to address potential sources of bias	16
29				
30				
31	Study size	#10	Explain how the study size was arrived at	9
32				
33	Quantitative	#11	Explain how quantitative variables were handled in the	7-9
34	variables		analyses. If applicable, describe which groupings were chosen,	
35			and why	
36				
37				
38	Statistical	#12a	Describe all statistical methods, including those used to control	9
39	methods		for confounding	
40				
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42				
43				
44	Statistical	#12b	Describe any methods used to examine subgroups and	9
45	methods		interactions	
46				
47				
48	Statistical	#12c	Explain how missing data were addressed	9
49	methods			
50				
51				
52	Statistical	#12d	If applicable, explain how loss to follow-up was addressed	9
53	methods			
54				
55				
56	Statistical	#12e	Describe any sensitivity analyses	9
57	methods			
58				
59				
60				

Results

Participants	#13a	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for exposed and unexposed groups if applicable.	9
Participants	#13b	Give reasons for non-participation at each stage	30
Participants	#13c	Consider use of a flow diagram	
Descriptive data	#14a	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	9,23-24
Descriptive data	#14b	Indicate number of participants with missing data for each variable of interest	9,23-26
9,23-26			
Descriptive data	#14c	Summarise follow-up time (eg, average and total amount)	4, 10
Outcome data	#15	Report numbers of outcome events or summary measures over time. Give information separately for exposed and unexposed groups if applicable.	11
Main results	#16a	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
Main results	#16b	Report category boundaries when continuous variables were categorized	10
Main results	#16c	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11

Other analyses	#17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11-13
Discussion			
Key results	#18	Summarise key results with reference to study objectives	13-14
Limitations	#19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	16
Interpretation	#20	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	15-16
Generalisability	#21	Discuss the generalisability (external validity) of the study results	16-17
Other Information			
Funding	#22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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